Université de Montréal

# Effet préventif de la milrinone inhalée chez les patients avec hypertension pulmonaire subissant une chirurgie cardiaque sous circulation extracorporelle: une approche pharmacométrique

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# Résumé

La circulation extracorporelle (CEC) est une technique utilisée en chirurgie cardiaque effectuée des milliers de fois chaque jour à travers le monde. L'instabilité hémodynamique associée au sevrage de la CEC difficile constitue la principale cause de mortalité en chirurgie cardiaque et l'hypertension pulmonaire (HP) a été identifiée comme un des facteurs de risque les plus importants. Récemment, une hypothèse a été émise suggérant que l'administration prophylactique (avant la CEC) de la milrinone par inhalation puisse avoir un effet préventif et faciliter le sevrage de la CEC chez les patients atteints d'HP. Toutefois, cette indication et voie d'administration pour la milrinone n'ont pas encore été approuvées par les organismes réglementaires. Jusqu'à présent, la recherche clinique sur la milrinone inhalée s'est principalement concentrée sur l'efficacité hémodynamique et l'innocuité chez les patients cardiaques, bien qu'aucun biomarqueur n'ait encore été établi. La dose la plus appropriée pour l'administration par nébulisation n'a pas été déterminée, de même que la caractérisation des profils pharmacocinétiques (PK) et pharmacodynamiques (PD) suite à l'inhalation. L'objectif de notre recherche consistait à caractériser la relation exposition-réponse de la milrinone inhalée administrée chez les patients subissant une chirurgie cardiaque sous CEC.

Une méthode analytique par chromatographie liquide à haute performance couplée à un détecteur ultraviolet (HPLC-UV) a été optimisée et validée pour le dosage de la milrinone plasmatique suite à l'inhalation et s'est avérée sensible et précise. La limite de quantification (LLOQ) était de 1.25 ng/ml avec des valeurs de précision intra- et inter-dosage moyennes (CV%) <8%. Des patients souffrant d'HP pour lesquels une chirurgie cardiaque sous CEC était prévue ont d'abord été recrutés pour une étude pilote (n=12) et, par la suite, pour une étude à plus grande échelle (n=28) où la milrinone (5 mg) était administrée par inhalation pré-CEC. Dans l'étude pilote, nous avons comparé l'exposition systémique de la milrinone peu après son administration avec un nébuliseur pneumatique ou un nébuliseur à tamis vibrant. L'efficacité des nébuliseurs en termes de dose émise et dose inhalée a également été déterminée *in vitro*. Dans l'étude à plus grande échelle conduite en utilisant exclusivement le nébuliseur à tamis vibrant, la dose

inhalée *in vivo* a été estimée et le profil pharmacocinétique de la milrinone inhalée a été pleinement caractérisé aux niveaux plasmatique et urinaire. Le ratio de la pression artérielle moyenne sur la pression artérielle pulmonaire moyenne (PAm/PAPm) a été choisi comme biomarqueur PD. La relation exposition-réponse de la milrinone a été caractérisée pendant la période d'inhalation en étudiant la relation entre l'aire sous la courbe de l'effet (ASCE) et l'aire sous la courbe des concentrations plasmatiques (ASC) de chacun des patients. Enfin, le ratio PAm/PAPm a été exploré comme un prédicteur potentiel de sortie de CEC difficile dans un modèle de régression logistique.

Les expériences *in vitro* ont démontré que les doses émises étaient similaires pour les nébuliseurs pneumatique (64%) et à tamis vibrant (68%). Cependant, la dose inhalée était 2-3 fois supérieure (46% vs 17%) avec le nébuliseur à tamis vibrant, et ce, en accord avec les concentrations plasmatiques. Chez les patients, en raison des variations au niveau des facteurs liés au circuit et au ventilateur causant une plus grande dose expirée, la dose inhalée a été estimée inférieure (30%) et cela a été confirmé après récupération de la dose de milrinone dans l'urine 24 h (26%). Les concentrations plasmatiques maximales ($C_{max}$: 41-189 ng/ml) et l'ampleur de la réponse maximale $\Delta_{Rmax-R0}$ (0-65%) ont été observées à la fin de l'inhalation (10-30 min). Les données obtenues suite aux analyses PK sont en accord avec les données publiées pour la milrinone intraveineuse. Après la période d'inhalation, les ASCE individuelles étaient directement reliées aux ASC (P=0.045). Enfin, notre biomarqueur PD ainsi que la durée de CEC ont été identifiés comme des prédicteurs significatifs de la sortie de CEC difficile.

La comparaison des ASC et ASCE correspondantes a fourni des données préliminaires supportant une preuve de concept pour l'utilisation du ratio PAm/PAPm comme biomarqueur PD prometteur et justifie de futures études PK/PD. Nous avons pu démontrer que la variation du ratio PAm/PAPm en réponse à la milrinone inhalée contribue à la prévention de la sortie de CEC difficile.

**Mots-clés** : chirurgie cardiaque; circulation extracorporelle; hypertension pulmonaire; inhalation; milrinone; nébuliseur; patients; pharmacocinétique; pharmacodynamie.

# Abstract

Cardiopulmonary bypass (CPB) is a technique used during cardiac surgery performed thousands of times each day worldwide. Hemodynamic complications associated with difficult separation from CPB represent a leading cause of mortality in cardiac surgery and pulmonary hypertension (PH) was identified as one of the most important predictor and risk factor. Recently, inhaled milrinone administration prior to CPB was hypothesized to have a preventive effect and facilitate separation from CPB in patients with PH. However, this indication and route of administration have not yet been approved by regulatory agencies for milrinone. So far, research efforts on inhaled milrinone have mainly focused on evidence supporting hemodynamic efficacy and safety in cardiac patients although no biomarker has been established. The most appropriate dose for nebulization has never been determined, nor have pharmacokinetic (PK) and pharmacodynamic (PD) profiles been characterized after inhalation. The objective of the current research consisted of characterizing the exposure-response relationship for milrinone administered by inhalation in patients undergoing cardiac surgery.

An improved high-performance liquid chromatography (HPLC) analytical assay using UV detection was validated for the quantification of milrinone in plasma after inhalation and proved to be sensitive and accurate. The lower limit of quantification (LLOQ) was 1.25 ng/ml with mean intra-assay and inter-assay precisions (CV%) <8%. Pulmonary hypertensive patients scheduled for cardiac surgery with CPB were first recruited for a pilot (n=12) and, subsequently, a full-scale (n=28) study where milrinone (5mg) was administered by inhalation pre-CPB. In the pilot study, milrinone early systemic exposure was investigated using a jet nebulizer or a mesh nebulizer. Nebulizers performance in terms of emitted and inhaled doses were also determined *in vitro*. In the full-scale study, using a mesh nebulizer exclusively, *in vivo* inhaled dose was estimated and milrinone definite pharmacokinetics fully characterized based on blood sampling and urine collection. Mean arterial pressure to mean pulmonary arterial pressure ratio (mAP/mPAP) was selected as the PD biomarker. Milrinone exposure-response

relationship was characterized during the inhalation period by studying the relationship between individual area under the effect-time curve (AUEC) and corresponding area under the plasma concentration-time curve (AUC). Finally, the mAP/mPAP ratio, among other variables, was explored as a potential predictor of difficult separation from bypass in a multiple logistic regression model.

*In vitro* experiments demonstrated that emitted doses were similar for the jet (64%) and the mesh (68.0%) nebulizers. However, the inhaled dose was 2-3 fold higher (46% vs 17%) after mesh nebulization, which was in agreement with plasma concentrations. In patients, due to variations in circuit-related and ventilator-related factors, the inhaled dose was estimated to be lower (30%) and this was confirmed by 24-h recovery in urine (26%). Milrinone peak plasma concentrations ($C_{max}$: 41-189 ng/ml) and magnitude of peak response $\Delta_{Rmax-R0}$ (0-65%) were observed at the end of inhalation (10-30 min). Data obtained from PK analysis agreed with published data for intravenous milrinone. After the inhalation period, individual AUEC were directly related to AUC ($P$=0.045). Finally, our PD biomarker, expressed as $\Delta_{Rmax-R0}$, as well as CPB duration, were both identified as significant predictors of DSB.

The comparison of corresponding AUC and AUEC provided preliminary evidence of a proof of concept for the use of the mAP/mPAP ratio as a promising PD biomarker and warrants future PK/PD studies. Indeed, mAP/mPAP ratio variation in response to inhaled milrinone was found to contribute in the prevention of DSB.

**Keywords** : cardiac surgery; cardiopulmonary bypass; inhalation; milrinone; nebulizer; patients; pharmacokinetics; pharmacodynamic; pulmonary hypertension.

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# Abréviations

| Symbole | Unité | Définition |
|---|---|---|
| $A$ | $m^2$ | Aire |
| $A$, $B$, $\alpha$ et $\beta$ | --- | Macro-constantes pharmacocinétiques |
| ADME | --- | Absorption, distribution, métabolisme, élimination |
| AMP(c) | --- | Adénosine monophosphate (cyclique) |
| ASC | $ng \cdot ml^{-1} \cdot min$ | Aire sous la courbe de la concentration vs. temps |
| ASCE | $u \cdot min$ | Aire sous la courbe de l'effet vs. temps |
| ATP | --- | Adénosine triphosphate |
| AUC | $ng \cdot ml^{-1} \cdot min$ | Area under the concentration vs. time curve,<br>*Aire sous la courbe de la concentration vs. temps* |
| AUEC | $u \cdot min$ | Area under the effect vs. time curve,<br>*Aire sous la courbe de l'effet vs. temps* |
| AVR | --- | Aortic valve replacement |
| $C$ | ng/ml | Concentration de médicament |
| $C_{éq}$ | ng/ml | Concentration plasmatique à l'état d'équilibre |
| $C_{last}$ | ng/ml | Concentration du dernier prélèvement |
| $C_{max}$ | ng/ml | Concentration plasmatique maximale |
| $\hat{C}_p$ | ng/ml | Concentrations prédites |
| $C_p$ | ng/ml | Concentrations plasmatiques |
| CABG | --- | Coronary artery bypass graft surgery |
| cAMP | --- | Adénosine monophosphate cyclique |
| CEC | --- | Circulation extracorporelle |
| CI | --- | Cardiac index,<br>*Index cardiaque* |
| $Cl$ | L/h | Clairance systémique ou totale |
| $Cl_R$ | L/h | Clairance rénale |
| CME | ng/ml | Concentration minimale efficace |
| CMT | ng/ml | Concentration maximale tolérée |
| CO | --- | Cardiac output,<br>*Débit cardiaque* |

| | | |
|---|---|---|
| CPB | --- | Cardiopulmonary bypass,<br>*Circulation extracorporelle* |
| CV | % | Coefficient de variation |
| D | --- | Diastolic,<br>*Diastolique* |
| $D$ | --- | Dose |
| $D_{inh}$ | --- | Dose inhalée |
| $D_{iv}$ | --- | Dose intraveineuse |
| $e^{-\alpha t}$ | --- | Phase de distribution |
| $e^{-\beta t}$ | --- | Phase d'élimination |
| $E$ | --- | Coefficient d'extraction |
| $E$ | --- | Effet pharmacologique |
| $E_0$ | --- | Effet observé à l'état basal |
| $E_{max}$ | --- | Effet maximal |
| $EC_{50}$ | ng/ml | Concentration produisant 50% de l'effet maximal |
| *ELS* | --- | Extended least squares,<br>*Critère des moindres carrés étendus* |
| ET-1 | --- | Endothéline-1 |
| ETO | --- | Échographie transoesophagienne |
| $F$ | --- | Biodisponibilité |
| FDA | --- | U.S. Food and Drug Administration |
| *GLS* | --- | Generalized least squares,<br>*Critère des moindres carrés généralisés* |
| Gs | --- | Protéine G |
| GTP | --- | Guanosine triphosphate |
| HP | --- | Hypertension pulmonaire |
| HPLC | --- | High-performance liquid chromatography,<br>*Chromatographie liquide à haute performance* |
| ICM | --- | Institut de Cardiologie de Montréal |
| ICU | --- | Intensive Care Unit,<br>*Soins intensifs* |
| IL-6 | --- | Interleukine-6 |
| IE | --- | Indice d'excentricité |

| | | |
|---|---|---|
| IV | --- | Intravenous,<br>*Intraveineux* |
| iMil | --- | Inhaled milrinone,<br>*Milrinone inhalée* |
| iNO | --- | Inhaled nitric oxide,<br>*Oxyde nitrique inhalée* |
| $iPGI_2$ | --- | Inhaled prostacyclin,<br>*Prostacycline inhalée* |
| $k_e$ | $min^{-1}$ | Constante de vitesse d'élimination |
| $k_{xx}$ | | Constantes de vitesse |
| $k_{10}$ | $min^{-1}$ | Constante de vitesse d'élimination |
| $k_{12}$, $k_{21}$ | $min^{-1}$ | Constantes de vitesse de transfert |
| LLOQ | ng/ml | Lower limit of quantification,<br>*Limite inférieure de quantification* |
| Log P | --- | Coefficient de partage octanol/eau |
| LV | --- | Left ventricule,<br>*Ventricule gauche* |
| mAP | mmHg | Mean arterial pressure,<br>*Pression artérielle moyenne* |
| MMAD | µm | Mass median aerodynamic diameter,<br>*Diamètre aérodynamique médian en masse* |
| mPAP | mmHg | Mean pulmonary artery pressure,<br>*Pression artérielle pulmonaire moyenne* |
| MTC | ng/ml | Concentration maximale tolérée |
| OAT, OATP, OCT | --- | Transporteurs organiques membranaires |
| OF | --- | Fonction objective,<br>*Objective function* |
| *OLS* | --- | Ordinary least squares,<br>*Critère des moindres carrés ordinaires* |
| *P* | --- | Perméabilité membranaire |
| Pa | mmHg | Arterial pressure,<br>*Pression artérielle* |
| PAOP | mmHg | Pulmonary artery occlusion pressure,<br>*Pression artérielle pulmonaire d'occlusion* |
| PAm | mmHg | Pression artérielle moyenne |
| PAP | mmHg | Pressions artérielles pulmonaires |

| | | |
|---|---|---|
| PAPm | mmHg | Pression artérielle pulmonaire moyenne |
| PCWP | mmHg | Pulmonary capillary wedge pressure,<br>*Pression artérielle pulmonaire d'occlusion* |
| PD | --- | Pharmacodynami(qu)e |
| PDE3 | --- | Phosphodiestérase de type 3 |
| PH | --- | Pulmonary hypertension,<br>*Hypertension pulmonaire* |
| PK | --- | Pharmacocinétique |
| pKa | --- | Constante de dissociation |
| PM | g/mol | Poids moléculaire |
| pMDIs | --- | Pressurized metered-dose inhalers,<br>*Inhalateurs-doseurs pressurisés* |
| Ppa | mmHg | Pulmonary artery pressure,<br>*Pression artérielle pulmonaire* |
| PPM | --- | Patient-prosthesis-mismatch,<br>Incohérence patient-prothèse |
| Pra | mmHg | Right atrial pressure,<br>*Pression auriculaire droite* |
| Prv | mmHg | Right ventricular pressure,<br>*Pression ventriculaire droite* |
| PVR | $dyn \cdot sec \cdot cm^{-5}$ | Pulmonary vascular resistance,<br>*Résistance pulmonaire vasculaire* |
| PVRI | $dyn \cdot sec \cdot cm^{-5} \cdot m^{-2}$ | Pulmonary vascular resistance index,<br>*Index de résistance pulmonaire vasculaire* |
| $Q$ | L/h | Clairance de distribution |
| $R_0$ | --- | Ratio PAm/PAPm au temps zéro |
| $R_{max}$ | --- | Ratio PAm/PAPm maximal |
| $R_{post\text{-}CEC}$ | --- | Ratio PAm/PAPm post-CEC |
| RA | --- | Right atrium,<br>*Oreillette droite* |
| ROC | --- | Receiver operating characteristics,<br>*Caractéristique opératoire du receveur* |
| RV | --- | Right ventricule,<br>*Ventricule droit* |
| RVP | --- | Résistances vasculaires pulmonaires |

| | | |
|---|---|---|
| S | --- | Systolic,<br>*Systolique* |
| SPAP | mmHg | Systolic pulmonary artery pressure,<br>*Pression artérielle pulmonaire systolique* |
| SVRI | $dyn \cdot sec \cdot cm^{-5} \cdot m^{-2}$ | Systemic vascular resistance index,<br>*Index de résistance vasculaire systémique* |
| $t$ | h | Temps |
| $t_0$ | h | Temps zéro |
| $t_{1/2}$ | h | Temps de demi-vie d'élimination apparente |
| $t_{1/2\ \alpha}$ | | Temps de demi-vie de distribution |
| $t_{1/2\ \beta}$ | | Temps de demi-vie d'élimination |
| $t_{last}$ | h | Temps du dernier prélèvement |
| $t_{max}$ | h | Temps auquel $C_{max}$ est observée |
| TEE | --- | Transesophageal echocardiography,<br>*Échocardiographie transoesophagienne* |
| THC | --- | Δ-9-tetrahydrocannabinol |
| TNF-α | --- | Facteur de nécrose tumorale alpha |
| UV | --- | Ultraviolet |
| $v_{abs}$ | min | Vitesse d'absorption |
| $V_{d,\ x}$ | L | Volume apparent de distribution |
| $V_1$ | L | Volume apparent de distribution central |
| $V_2$ | L | Volume apparent du compartiment périphérique |
| $W$ | --- | Weight,<br>*Pondération* |
| WHO | --- | The World Health Organization |
| *WLS* | --- | Weighted least squares,<br>*Critère des moindres carrés pondérés* |
| $\lambda_z$ | $min^{-1}$ | Constante de vitesse d'élimination |
| $\Delta t$ | min | Intervalle de temps entre deux prélèvements |
| $\Delta_{Rmax\text{-}R0}$ | --- | Amplitude de la réponse maximale |

*À mes parents qui m'ont tout donné,*

*nhờ Bố & Mẹ đã cho con tất cả.*

# Remerciements


*À ma directrice et mon codirecteur de recherche,*

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# Avant-propos

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L'ouvrage qui suit se subdivise en trois grandes sections: une introduction générale (Section I), suivie des travaux de recherche (Section II), et une conclusion générale (Section III). La première section couvre une introduction aux principes de la circulation extracorporelle et les notions relatives à l'hypertension pulmonaire en chirurgie cardiaque (extraits du chapitre de livre dont je suis premier auteur), un rappel des concepts de base en pharmacocinétique et en pharmacodynamie, une mise à jour sur la pharmacologie clinique de la milrinone chez l'adulte, une revue des particularités de l'administration de médicaments par voie pulmonaire, ainsi que des principes de modélisation utilisés à des fins de caractérisation de l'effet clinique. La deuxième section est composée des trois articles scientifiques découlant des travaux de recherche. Finalement, la dernière section consiste en une discussion générale des résultats et des perspectives de recherche.

# SECTION I: INTRODUCTION

# CHAPITRE 1. Chirurgie cardiaque sous circulation extracorporelle

## 1.1. Enjeux et société

Au Canada, le taux de mortalité liée à une cause cardiovasculaire a diminué de plus de 75 pour cent depuis les soixante dernières années et près de 40 pour cent de cette diminution est survenue durant la dernière décennie seulement. Ce progrès résulte, en grande partie, de l'avancement de la recherche au niveau des traitements pharmacologiques, des techniques chirurgicales, ainsi que des stratégies préventives.[1]

Par ailleurs, dans le domaine de la chirurgie cardiaque, un changement démographique important a été observé; les patients sont plus âgés, plus malades et les procédures sont devenues plus complexes. En 2012, le nombre de personnes âgées (65 ans et plus) avait plus que doublé par rapport à il y a 30 ans, avec un Canadien sur deux âgé d'au moins 40 ans (la population québécoise étant légèrement plus âgée que le reste du Canada). Les projections démographiques les plus récentes indiquent que ce phénomène devrait s'accélérer au cours des 20 prochaines années avec une proportion de personnes âgées pouvant représenter plus du quart de la population canadienne à partir de 2036.[2]

Or, ce phénomène du vieillissement de la population exerce un impact significatif sur notre système de soins de santé. Une population plus âgée subissant une chirurgie cardiaque est beaucoup plus vulnérable, i.e. exposée à un plus grand risque de complications per- et postopératoires, ce qui a pour conséquence d'entraîner un besoin accru en soins avancés et des coûts supplémentaires.

## 1.2. Principes d'une circulation extracorporelle

Au Canada, environ 36 000 chirurgies cardiaques sont effectuées chaque année et presque toutes les procédures nécessitent l'utilisation d'une circulation extracorporelle (CEC). La CEC (Figure **1**), souvent désignée comme «pompe» ou cœur-poumon artificiel, est une technique qui permet temporairement d'assurer la fonction du cœur et des poumons, alors que ces organes sont contournés durant la procédure chirurgicale. Le fonctionnement optimal de l'ensemble des composantes du circuit de la CEC est assuré

par un perfusionniste clinique travaillant en étroite collaboration avec le chirurgien et l'anesthésiste afin de maintenir une perfusion et une oxygénation adéquates de l'organisme tout au long de l'intervention. Ainsi, la CEC est rapidement devenue un outil indispensable en chirurgie cardiaque pour les simples raisons suivantes: il est plus difficile d'exécuter des interventions chirurgicales sur un cœur battant et certaines procédures, comme la chirurgie valvulaire, nécessitent l'ouverture obligatoire des cavités cardiaques.

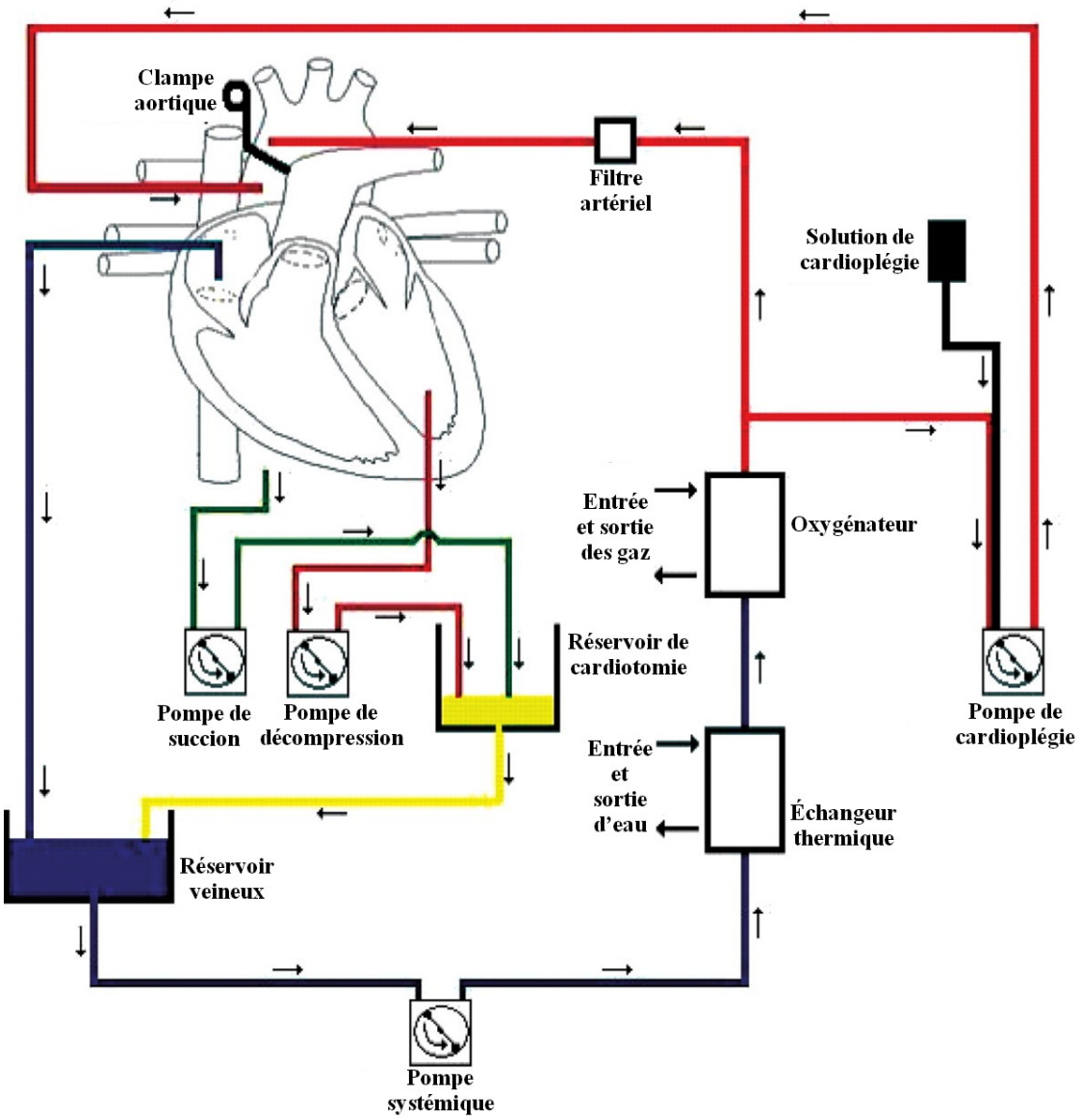


**Figure 1**. Représentation schématique d'un circuit de circulation extracorporelle. Adaptée de Machin.[3]

L'installation de la CEC est effectuée par un chirurgien cardiaque et consiste à insérer une canule artérielle dans l'aorte ascendante et une canule veineuse dans l'oreillette droite et/ou la veine cave. Ainsi, au niveau du circuit principal de la CEC, le sang veineux de la périphérie est drainé du corps du patient via la canule veineuse (reliée à un tube rempli d'une solution cristalloïde isotonique) et dirigé vers le réservoir veineux. À partir de ce réservoir, le sang est pompé vers l'oxygénateur en passant par un échangeur thermique pour être refroidi, oxygéné et filtré juste avant d'être retourné dans l'organisme via la canule artérielle. D'autres composantes importantes constituent également les circuits secondaires de la CEC. Il y a d'abord les circuits de succion « sucker » et de décompression « vent » qui assurent respectivement le retour du sang extra- et intracardiaque du champ opératoire vers le réservoir veineux. Enfin, le circuit de cardioplégie sert à préserver le cœur et maintenir le silence électrique. Afin d'éviter la coagulation sanguine durant la CEC, l'héparine est administrée au patient avant le démarrage de la procédure. Pendant la chirurgie cardiaque, l'hypothermie est souvent maintenue visant une température corporelle de 28 à 32°C dans le but de ralentir le métabolisme de base de l'organisme et diminuer son besoin en oxygène. Le sang refroidi durant la CEC a généralement une viscosité plus élevée que le sang à température corporelle normale, mais la solution cristalloïde utilisée pour amorcer la tubulure à l'installation de la CEC contribue à diluer le sang et diminuer sa viscosité. Une fois la chirurgie terminée, le sulfate de protamine est administré pour renverser l'effet de l'héparine avant le sevrage de la CEC.

## 1.3. Impacts physiologiques de la CEC

Bien que la CEC soit utilisée en chirurgie cardiaque depuis plus d'un demi-siècle et fonctionne avec succès plusieurs milliers de fois chaque jour à travers le monde, la procédure est associée à de nombreux inconvénients (e.g. saignements excessifs, inflammation systémique, accident vasculaire cérébral, dysfonction neuropsychologique, rénale, pulmonaire, cardiaque et défaillance multiviscérale). Tout au long de la CEC, le sang du patient se trouve exposé à la surface artificielle du circuit de CEC (tubulure, membranes, etc.), ce qui a pour conséquence de provoquer une réaction inflammatoire

secondaire à plusieurs mécanismes physiologiques: activation de l'endothélium, de la cascade du complément, des neutrophiles, de la thrombine et des plaquettes.[4]

Les poumons comptent parmi les organes les plus affectés par la CEC, alors qu'ils sont contournés tout au long de la procédure sans recevoir ni perfusion ni protection spécifique (contrairement au cœur qui reçoit une solution cardioplégique). Suite à la CEC, une dysfonction endothéliale pulmonaire entraîne souvent le syndrome de reperfusion pulmonaire et peut se manifester sous forme d'hypertension pulmonaire (HP) chez plusieurs patients. Le syndrome post-CEC est caractérisé par une augmentation de la perméabilité capillaire semblable au syndrome de détresse respiratoire aiguë chez l'adulte; il entraîne une réduction de l'apport en oxygène, une augmentation du gradient alvéolo-artériel, une baisse de compliance pulmonaire et une augmentation des résistances vasculaires pulmonaires. Au niveau de l'endothélium, une réduction de la synthèse d'oxyde nitrique[5, 6] et/ou une activation du système de l'endothéline (ET-1) prédisposent les patients à développer une HP suite à la CEC.[7-9] Par ailleurs, l'augmentation des niveaux d'ET-1 est corrélée avec la durée de CEC, la sévérité de l'HP et la dysfonction myocardique post-CEC,[10] ce qui justifie pourquoi une CEC de longue durée représente un facteur de risque important en chirurgie cardiaque.

Sans être nécessairement cliniquement significative, une détérioration de la fonction pulmonaire est invariablement observée chez les patients subissant une chirurgie cardiaque sous CEC. Plusieurs études ont démontré que les effets délétères exercés par la CEC sur les poumons[11-16] peuvent persister pendant plus de 3 mois après la chirurgie.[17-20] Or, la CEC peut représenter un défi de taille chez les patients atteints de problèmes pulmonaires avant la procédure, puisque ceux-ci sont encore plus à risque de développer des complications pulmonaires postopératoires, e.g. l'aggravation d'une HP déjà présente chez le patient avant l'opération (situation courante en chirurgie cardiaque).

## 1.4. Sevrage de CEC difficile

À la fin de la procédure chirurgicale, le sevrage de la CEC consiste à graduellement rétablir la circulation sanguine du cœur et des poumons afin de leur permettre de reprendre leurs fonctions normales. Or, chez certains patients, une aggravation de l'HP causée par le syndrome de reperfusion pulmonaire complique fréquemment le sevrage de la CEC. Quand ce phénomène se produit, la reprise des activités vitales de ces organes est difficile et nécessite l'administration d'agents vasoactifs et/ou le retour sous CEC pour pallier à cette dysfonction cardiaque (instabilité hémodynamique). Cette situation est qualifiée comme étant un sevrage de CEC difficile. Le sevrage de CEC difficile est un des facteurs de risque de mortalité les plus importants en chirurgie cardiaque.[21, 22] Une classification exclusive selon le niveau de difficulté du sevrage de la CEC a été dérivée suite à l'analyse des données de 6120 patients basée sur le(s) type(s) de support nécessaire(s) entre la fin de la CEC et la fin de la chirurgie (Tableau **I**).[23, 24] Ce support peut être de type pharmacologique tel que l'administration d'un vasopresseur (noradrénaline, phényléphrine, vasopressine) ou d'un inotrope (dobutamine, milrinone, adrénaline) ou de type non pharmacologique tel qu'un retour sous CEC suite à l'échec du sevrage, l'installation d'un ballon intra-aortique ou d'un dispositif d'assistance ventriculaire.

**Tableau I**. Classification du niveau de difficulté du sevrage de la CEC.

| **Niveau de difficulté du sevrage de la CEC** | **Support(s) nécessaire(s)** |
|---|---|
| Facile | ≤1 vasopresseur ou inotrope |
| Difficile | ≥1 vasopresseur + ≥1 inotrope |
| Très difficile<br>(Complexe) | Retour sous CEC<br>ou installation d'un ballon intra-aortique<br>ou installation d'un dispositif d'assistance ventriculaire |

CEC, circulation extracorporelle. Information tirée de Denault.[24]

Parmi les facteurs de risque potentiellement réversibles associés au sevrage de CEC difficile, l'HP a été identifiée comme étant le plus significatif.[25, 26] La présence d'HP exerce un effet délétère sur la fonction cardiaque causant une augmentation de la post-charge ventriculaire droite et pouvant conduire à l'insuffisance cardiaque droite.[27] Or, une dysfonction ventriculaire droite est associée à un taux de mortalité en chirurgie cardiaque pouvant atteindre jusqu'à 37%.[28, 29]

# CHAPITRE 2. Manuscrit n°1 (extraits du chapitre de livre annexe I): A pathophysiological approach to understanding pulmonary hypertension in cardiac surgery

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## 2.1. Introduction

Pulmonary hypertension (PH) is associated with increased morbidity and mortality and is an important prognostic factor in cardiac surgery. As the average age and associated co-morbidities of cardiac surgical patients increase, the prevalence of PH is likely to rise. In this chapter, we will define PH, classify it on the basis of pathophysiological etiology, and suggest treatment therapies according to this classification. The importance of PH in cardiac surgery, its relationship to right ventricular dysfunction and preventive therapies will also be discussed. When applicable, we will draw from our clinical experience with PH to suggest strategies for the prevention of possible complications.

## 2.2. Definition of pulmonary hypertension

### 2.2.1. Hemodynamic parameters used in clinical settings

There are several hemodynamic parameters used in defining PH (Table **II**).[30] These definitions have been used in various studies.

### 2.2.2. Diagnosis in awake and anesthetized patients

Pulmonary hypertension is usually diagnosed prior to cardiac surgery in awake patients. The diagnosis is obtained either directly by cardiac catheterization or indirectly by using Doppler signals from transesophageal echocardiography (TEE) and using Bernoulli's equation.

### 2.2.3. Comparison of absolute and relative values in the assessment of pulmonary hypertension

Following the induction of general anesthesia, a reduction in both the systemic and the pulmonary artery pressures is observed. Consequently, using absolute values of systolic pulmonary artery pressure (sPAP) in defining PH would underestimate its severity. To address this issue, Robitaille *et al*. studied 1557 patients undergoing cardiac surgery.[31] In the 32 patients with preoperative PH, induction of general anesthesia resulted in a significant reduction in mean arterial pressure (mAP) and mean pulmonary artery pressure (mPAP) but the ratio of mAP/mPAP remained stable (Figure **2**). The normal value for this ratio is >4, and lower values can be used to quantify the severity of PH.

**Table II**. Definitions of Pulmonary Hypertension Used in Clinical Settings

| **Hemodynamic parameter** | **Normal value** | **Abnormal value** |
|---|---|---|
| Systolic pulmonary artery pressure (sPAP) | 15-30 mmHg | >30 or ≥40 mmHg |
| Mean pulmonary artery pressure (mPAP) | 9-16 mmHg | Moderate >18 mmHg<br>Significant >25 mmHg<br>Exercise-induced >30 mmHg |
| Pulmonary vascular resistance (PVR) = (mPAP – PAOP) X 80/CO | 60-120 dyn·sec·cm$^{-5}$ | Mild >125 dyn·sec·cm$^{-5}$<br>Moderate >200-300 dyn·sec·cm$^{-5}$<br>Severe >600 dyn·sec·cm$^{-5}$ |
| Indexed pulmonary vascular resistance (PVRI) = (mPAP – PAOP) X 80/CI | 250-340 dyn·sec·cm$^{-5}$·m$^{-2}$ | >340 dyn·sec·cm$^{-5}$·m$^{-2}$ |
| Pulmonary to systemic vascular resistance index (PVRI/SVRI) X 100% | ≤10% | >10% |
| Transpulmonary gradient (mPAP – PAOP) | ≤14 mmHg | >14 mmHg |
| Mean pulmonary to systemic pressure ratio (mPAP/mAP) X 100% | <25% | Moderate 33-50%<br>Severe >50% |
| Mean systemic to pulmonary pressure ratio (mAP/mPAP) | ≥4 | <4 |

CI: cardiac index; CO: cardiac output; mAP: mean arterial pressure; PAOP: pulmonary artery occlusion pressure; SVRI: indexed systemic vascular resistance. Adapted from Gomez.[30]

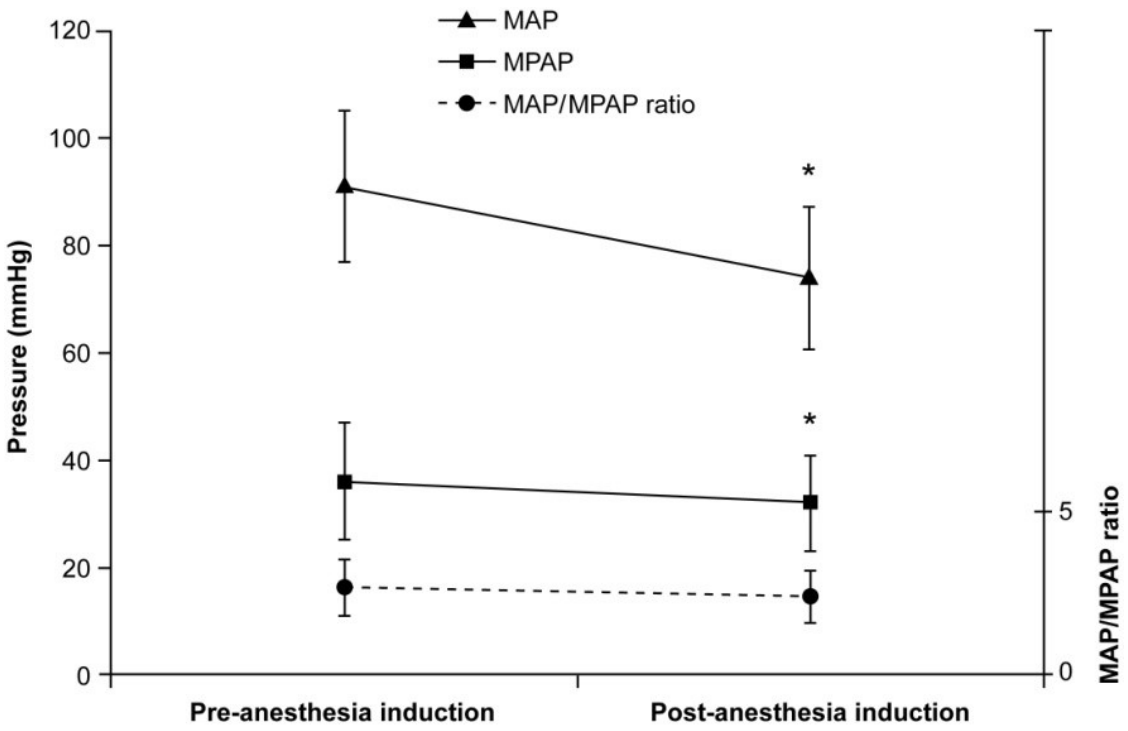


**Figure 2**. Changes in mean arterial pressure (mAP), mean pulmonary artery pressure (mPAP), and the mAP/mPAP ratio after the induction of anesthesia in 32 patients with preoperative pulmonary hypertension. No significant change in the mAP/mPAP ratio was observed (*p <0.05).[31]

The relevance of the mAP/mPAP ratio was demonstrated after comparing its ability to estimate the probability of postoperative complications with the ability of other normally used hemodynamic parameters for this purpose (listed in Table **II**). Values of the ratio obtained after induction of general anesthesia but before cardiopulmonary bypass (CPB) in 1439 patients undergoing cardiac surgery showed similar trend when compared to other hemodynamic parameters (Figure **3**). Furthermore, the ratio turned out to be the best predictor of perioperative complications, defined as death, need for intra-aortic balloon pump, cardiac arrest, or use of vasoactive support for more than 24 hours.

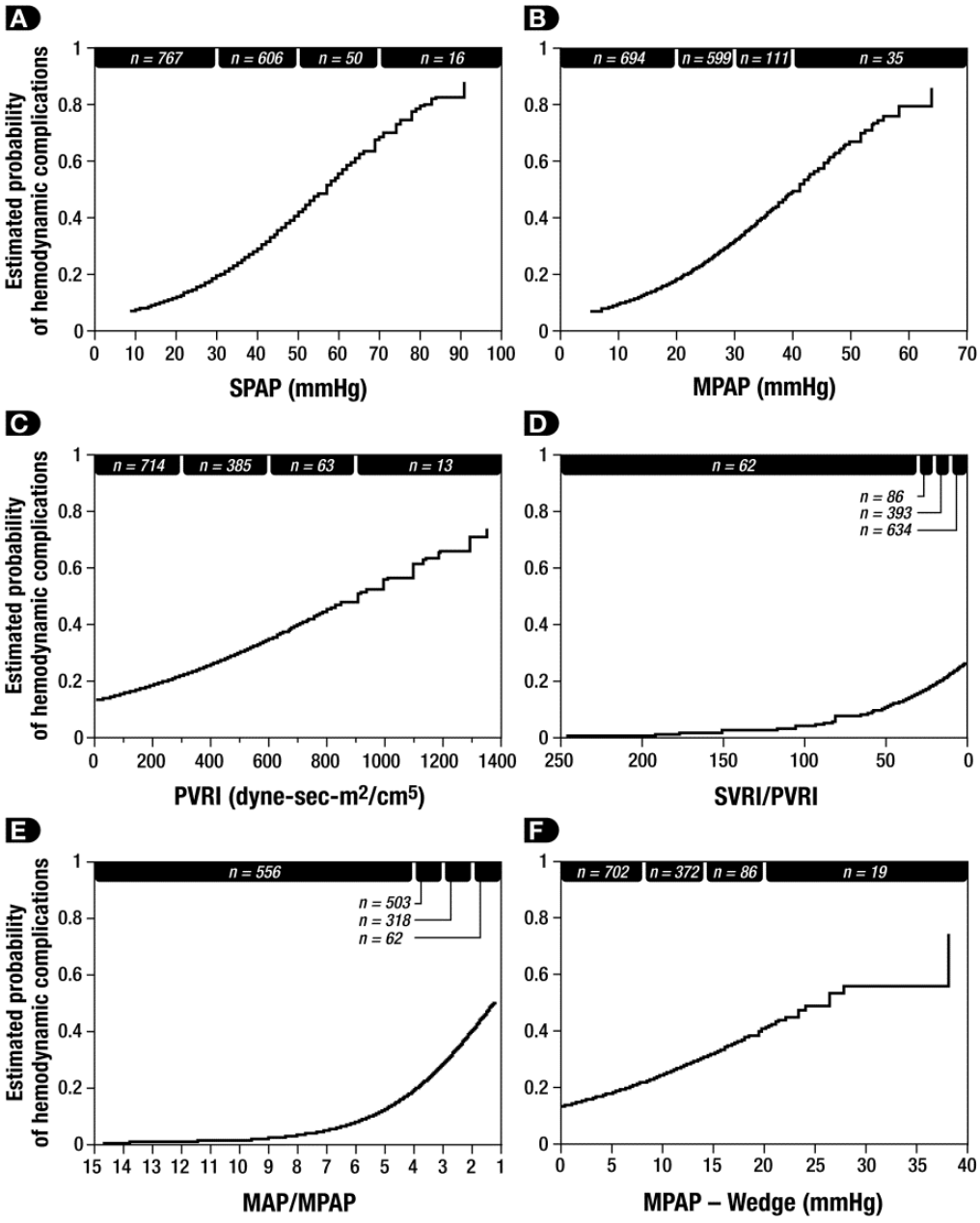


**Figure 3**. Relationship between the estimated probability of hemodynamic complications and variables used in the evaluation of pulmonary hypertension: (**A**) systolic pulmonary artery pressure (sPAP), (**B**) mean pulmonary artery pressure (mPAP), (**C**) indexed pulmonary vascular resistance (PVRI), (**D**) systemic to pulmonary vascular resistance index ratio (SVRI/PVRI), (**E**) mean systemic to pulmonary pressure ratio (mAP/mPAP), and (**F**) transpulmonary gradient defined as mPAP-Wedge or pulmonary artery occlusion pressure (PCWP or PAOP). For easier comparison, the scale of the x axis of the SVRI/PVRI and the mAP/mPAP are inverted. (n=number of patients).[31]

An abnormal mAP/mPAP ratio was also recognized to be significantly correlated with abnormal systolic and/or diastolic cardiac function (Figure **4**).[31] The use of relative instead of absolute values to estimate PH is currently used in congenital cardiology.[32, 33].

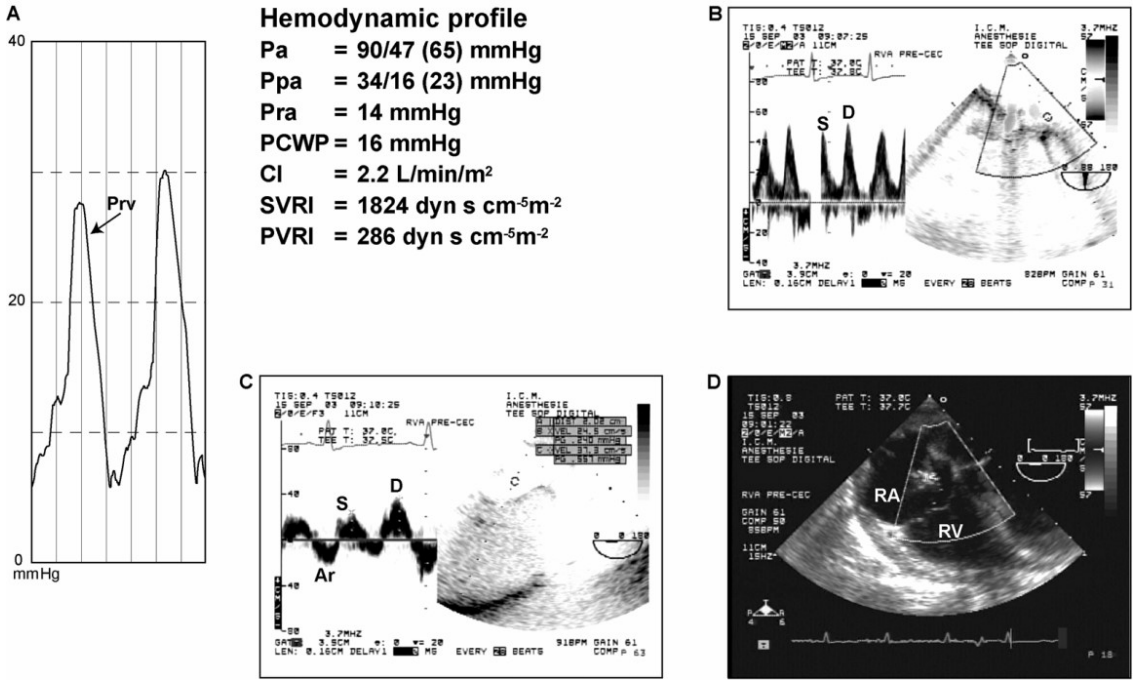


**Figure 4**. Hemodynamic and transesophageal echocardiographic evaluation of a 46-year-old woman scheduled for aortic valve surgery. Despite a normal pulmonary artery pressure (Ppa) of 34/16 mmHg and pulmonary vascular resistance index (PVRI) at 286 dyn·s·cm$^{-5}$·m$^{-2}$, this patient had an abnormal right ventricular diastolic filling pressure waveform characterized by a rapid upstroke (**A**) and reduced systolic (S) to diastolic (D) pulmonary (**B**) and hepatic (**C**) venous flows consistent with left and right ventricular diastolic dysfunction. In addition, a dilated right atrium and ventricle were present without significant tricuspid regurgitation in a mid-esophageal right ventricular view (**D**). The mean systemic to pulmonary pressure ratio (mAP/mPAP) ratio was 65/23 or 2.8. (CI: cardiac index; Pa: arterial pressure; PCWP: pulmonary capillary wedge pressure; Pra: right atrial pressure; Prv: right ventricular pressure; RA: right atrium; RV: right ventricle; SVRI: systemic vascular resistance index).[31]

In summary, the evaluation and diagnostic of PH in cardiac surgical patients must be done using specific criteria. In awake patients, the absolute values can be used since they correlate well with outcomes. However, in patients under general anesthesia, the ratio of mAP/mPAP allows to screen for PH when systolic blood pressures are lower due to the anesthetic agents.

## 2.3. Classification of pulmonary hypertension based on pathophysiology and etiology

The 2008 World Symposium on PH endorsed by The World Health Organization (WHO) proposed a classification system divided into 5 groups: 1) Pulmonary arterial hypertension, 2) PH owing to left heart disease, 3) PH owing to lung diseases and/or hypoxia, 4) Chronic thromboembolic PH, and 5) PH with unclear or multifactorial etiologies.[34] In cardiac surgery, PH is more frequently classified as pre-capillary, capillary or post-capillary, depending on the site where the underlying cause of PH is found. In this context, PH during cardiac surgery is typically post-capillary since the cause is mainly of left ventricular (LV) origin, past the pulmonary capillary bed.

The causes underlying PH in cardiac surgery can be complex and may result from several mechanisms acting alone or in combination (Figure **5**). These mechanisms may exist before the operation or appear during or after the procedure. Exacerbation of PH may happen at any time during cardiac surgery, before, during or after CPB. Indeed, patients are at risk of LV failure at all times, especially after CPB when the reperfusion of the ischemic lungs can cause pulmonary reperfusion syndrome. Finally, PH can persist postoperatively secondary to a patient-prosthesis-mismatch (PPM) after mitral or aortic valve replacement. The treatment of PH is based on the identification of its etiology, whence the importance of distinguishing between the different pathophysiologies.

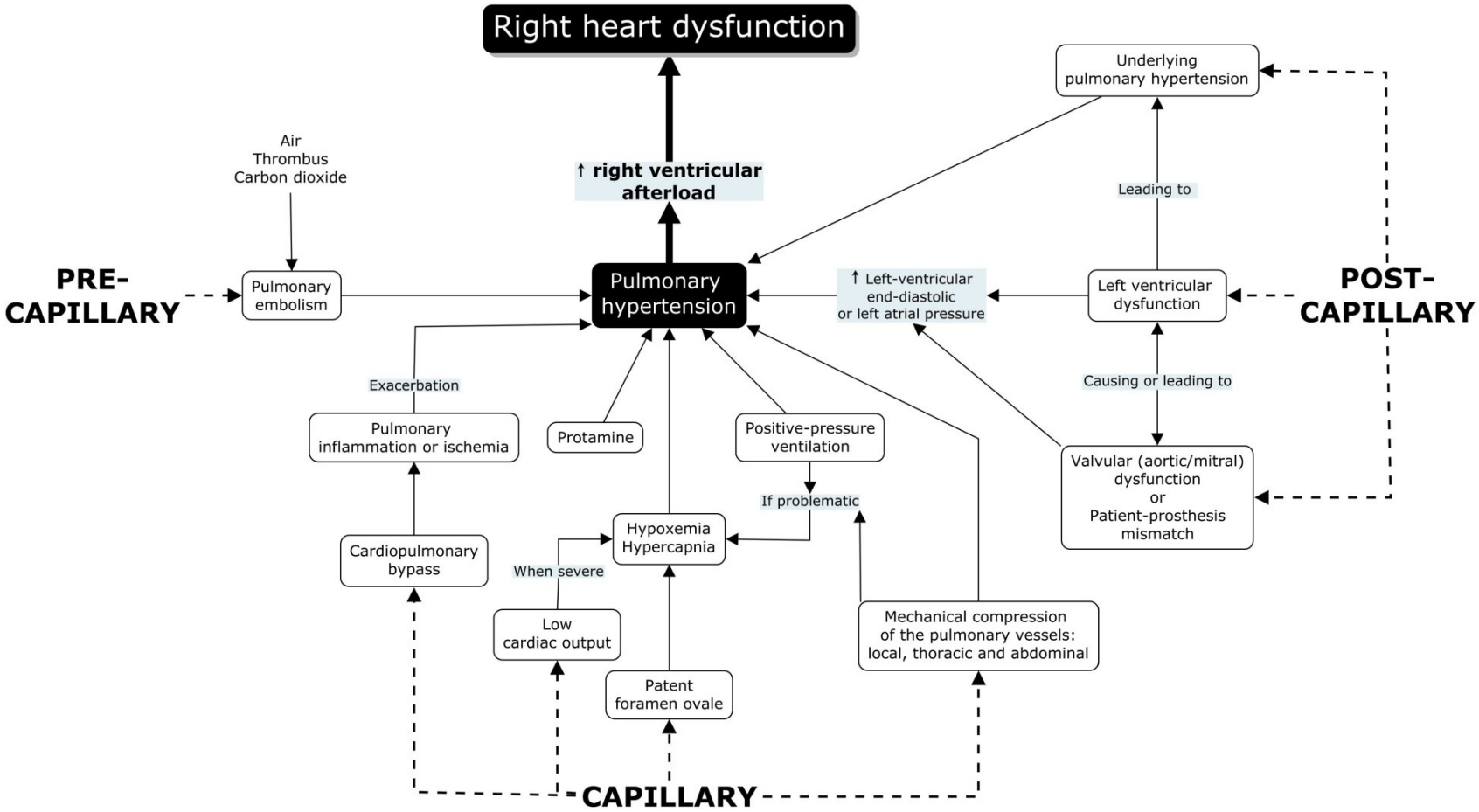


**Figure 5**. Major mechanisms of pulmonary hypertension in cardiac surgery. Other mechanisms may be operating at several levels: for instance, hypoxia (capillary) may lead to pulmonary hypertension, right ventricular systolic failure and, through interventricular interaction, left ventricular diastolic function (post-capillary).

## 2.3.1. Review of the factors involved

The most important causes of PH in cardiac surgery, illustrated in Figure **5**, are classified according to their originating anatomical site: pre-capillary, capillary and post-capillary.

### 2.3.1.1. Capillary: Cardiopulmonary bypass

Pulmonary damage during CPB is one of the important etiologies of PH in cardiac surgery. This is mainly due to the fact that the lungs are ischemic during CPB. The underlying mechanisms include 1) release of cytokines through endotoxin production,[35] 2) complement activation and 3) ischemia reperfusion injury[4, 36] which leads to the production of free radicals, endothelin and prostacyclin derivatives with nitric oxide inhibition.[4] The resulting systemic inflammatory response, pulmonary reperfusion syndrome as well as the transfusion of blood products may all exacerbate PH (Figure **6**).[37, 38]

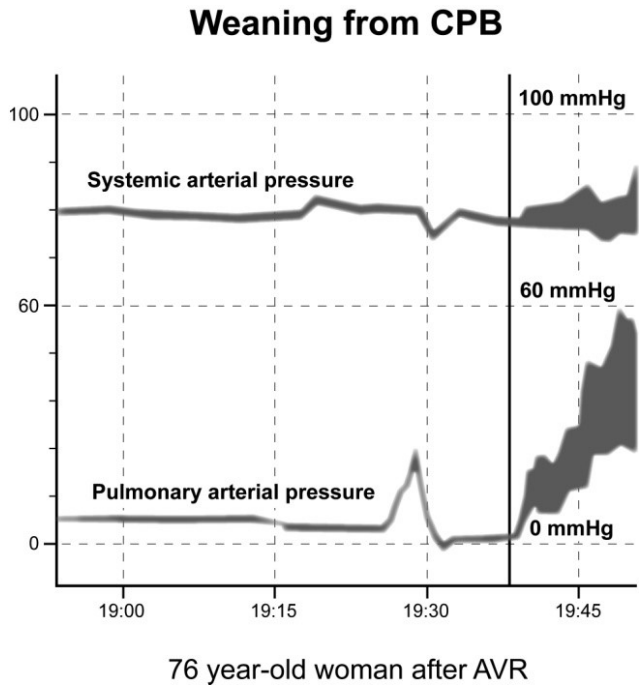


**Figure 6**. Unexpected pulmonary hypertension upon weaning from cardiopulmonary bypass (CPB) in a 76 year-old woman after aortic valve replacement (AVR). The CPB duration was 71 minutes. A significant increase in pulmonary arterial pressure in relation to the systemic arterial pressure was observed as the patient was weaned from CPB. No mechanical causes were found.

During CPB, blood is exposed to an artificial surface for oxygenation before it is sent back into the systemic circulation. This is associated with an inflammatory reaction secondary to endothelial activation, activation of the complement cascade, neutrophils, thrombin and platelets. Since the heart and lungs do not receive blood during CPB, cardioplegia solutions are used to preserve heart function, however, no specific protection is undertaken for the pulmonary circulation. In some patients, this may result in pulmonary reperfusion syndrome associated with postoperative endothelial dysfunction and PH or in post-CPB respiratory distress syndrome. The latter phenomenon, similar to the respiratory distress syndrome in adults, is characterized by an increased capillary permeability leading to a reduction in oxygenation, increased alveolar-arterial gradient, decreased lung compliance, increased pulmonary vascular resistance (PVR), and exacerbation of preoperative PH. Activation of the endothelin system during CPB increases endothelin (ET-1) concentrations and correlates with CPB duration, severity of PH and post-CPB myocardial dysfunction. For this reason, CPB duration plays a major role in the incidence of mortality in cardiac surgery. Post-CPB PH can lead to right ventricular (RV) dysfunction which, when severe, is fraught with a 44 to 86% mortality rate.

## 2.4. Treatment of pulmonary hypertension in cardiac surgery based on pathophysiology and etiology

The approach to pharmacological and non-pharmacological treatment of PH will be directed towards the cause or the consequence of PH, as illustrated in Figure **5**. Most often, treatment of the underlying mechanism causing PH requires non-pharmacological approaches, while pharmacological approaches will usually be the solution for the treatment of persisting PH and its consequence, RV failure.

### 2.4.1. Pharmacological and non-pharmacological approaches

Therapeutic management of PH has dramatically improved in the last years, offering both relief from symptoms and prolonged survival. However, there is still no cure for this disease. Moreover, in presence of PH, the choice of the appropriate therapy should rely on evidence-based medicine.

#### 2.4.1.1. Capillary: Cardiopulmonary bypass

As discussed, CPB causes pulmonary damage during surgery through different mechanisms, potentially leading to PH but, more frequently, it contributes to the exacerbation of PH caused by other factors during the surgical procedure. In this context, patients can benefit from PH-specific medical therapy and prophylactic treatments for PH use in cardiac surgery, which will be discussed later in this chapter.

### 2.4.2. Experience at the Montreal Heart Institute

At the Montreal Heart Institute, inhaled prostacyclin ($iPGI_2$)[39, 40] and inhaled milrinone[10, 41] are commonly administered to patients when PH and RV dysfunction occur before or after cardiac surgery. Oral sildenafil and inhaled nitric oxide (iNO) are also used in refractory cases in the intensive care unit. Administration by inhalation has the advantage of selectively reaching well-ventilated regions of the lung and thus avoiding undesired decreases of systemic pressures. Future strategies may include the combination of currently available drugs and improvement of methods of administration for current drugs.

## 2.5. Importance and impact of pulmonary hypertension in cardiac surgery

Preoperative PH is associated with increased morbidity and mortality in cardiac surgery.[25, 42-45] Therefore, awareness of PH is very important and its presence in any form should be routinely reported to the surgeon and be evaluated in risk stratification models. Yet, this is not always the case, since only 4/19 risk stratification models in cardiac surgery include preoperative PH as a risk factor.[46] Interestingly, PH is included in the EuroSCORE model which had the greatest discriminatory power over all other models. In a Swedish study including 4351 coronary artery bypass graft surgery (CABG) patients, the receiver operating characteristics (ROC) of the EuroSCORE model was found to be 0.86 and 0.75 for the 30-day and one year mortality rates, respectively.

Analysis performed using the Montreal Heart Institute anesthesia database in 1999 on a total of 1439 patients revealed a mean preoperative sPAP of 31±10 mmHg. PH was

defined as sPAP >30 mmHg and was observed in 605 patients (42%). The type of procedures performed in this subpopulation were mainly mitral valve replacements (*n*=80, 40±14 mmHg), followed by combined CABG and valve procedures (*n*=126, 36±13 mmHg), multiple valve procedures (*n*=60, 36±16 mmHg) and heart transplantations (*n*=6, 36±14 mmHg). Severe PH defined as mAP/mPAP ratio <2 was observed in 16 patients, who all experienced difficult separation from CPB, half of them required postoperative vasoactive support for more than 24 hours while 3 of them died (18.7%).

Thus, PH present before, during or after the operation has an impact on survival mostly through its deleterious effect on right-sided heart function. The most dreaded consequence of PH is the increase in RV afterload and RV dysfunction which will be addressed herein.

## 2.5.1. Right ventricular dysfunction

Regardless of the underlying cause, uncontrolled PH leads to RV dysfunction. There is growing evidence showing that morbidity and mortality associated with PH depends on RV adaptation to the disease rather than on the absolute values of pulmonary artery pressure (PAP).[47-51]

### 2.5.1.1. Before the procedure

In patients with severe aortic stenosis, Boldt et al.[52] demonstrated that preoperative RV dysfunction was associated with increased requirements for postoperative inotropic support.

### 2.5.1.2. After the procedure

Right ventricular failure after CPB is associated with a mortality rate ranging from 44% to 86%.[53] The incidence of acute refractory RV failure ranges from 0.04 to 0.1% after cardiac surgery. Acute refractory RV failure has also been reported in 2-3% patients after heart transplantation and in 20-30% patients receiving support from a LV assist device with a reported initial salvage rate as low as 25-30%.[38]

## 2.6. Prevention of pulmonary hypertension in cardiac surgery

### 2.6.1. Pharmacological

Prevention of PH represents a promising strategy to prevent RV failure, its most important consequence after cardiac surgery. To date, very few studies have addressed this issue and one of the potential avenues constitutes the prevention of the pulmonary reperfusion syndrome. In this regard, both $iPGI_2$[54] and inhaled milrinone[10] have been demonstrated to prevent CPB-induced endothelial dysfunction, in an animal model. A pilot randomized controlled trial conducted by Hache *et al.*[40] in patients with preoperative PH concluded that $iPGI_2$ was superior to placebo in reducing PH and was also associated with lower requirements for vasoactive support.

## 2.7. Conclusion

Pulmonary hypertension and its most dreaded consequence, RV dysfunction, are important mortality risk factors in cardiac surgery. Accordingly, all cardiac patients may benefit from early diagnosis and/or treatment prior to the surgical procedure. In patients with PH, further evaluation of potential alterations in the RV function would be relevant. Thus, future trials should prioritize in-depth exploration of preventive approaches in order to address the role of preemptive reduction of PH severity before cardiac surgery and to determine its impact on postoperative outcomes and survival improvement.

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# CHAPITRE 3. Milrinone en chirurgie cardiaque

En chirurgie cardiaque, l'HP résultant d'une sortie de CEC difficile exerce des conséquences graves sur la fonction ventriculaire droite pouvant imposer une situation potentiellement mortelle si non traitée. Par le fait même, des stratégies préventives telles que l'administration prophylactique de supports pharmacologiques peuvent représenter une avenue thérapeutique prometteuse afin d'éviter de telles complications et contribuer à réduire la morbidité et mortalité en chirurgie cardiaque. Plusieurs traitements ont été étudiés dans le but de contrôler l'HP, particulièrement dans le contexte d'une sortie de CEC difficile en chirurgie cardiaque. Parmi les médicaments les plus utilisés de la grande classe des vasodilatateurs, la milrinone constitue un agent de choix.[55]

## 3.1. Mécanisme d'action

La milrinone est un inhibiteur sélectif de l'isoenzyme adénosine monophosphate cyclique (AMPc) phosphodiestérase de type III et correspond au médicament le plus fréquemment utilisé de cette catégorie en chirurgie cardiaque.[56] Son mécanisme d'action (Figure **7**) consiste à inhiber sélectivement l'activité de cette phosphodiestérase, ce qui entraîne une augmentation des concentrations intracellulaires d'AMPc au niveau des muscles lisses cardiaques et vasculaires. Ceci résulte en une augmentation du calcium intracellulaire sous forme ionisée qui renforce la contractilité myocardique ainsi qu'en une phosphorylation de la protéine contractile AMPc-dépendante qui accentue la vasodilatation.

Ainsi, la milrinone est un inodilatateur (inotrope positif et vasodilatateur) dont les effets cliniques se traduisent par une augmentation du débit cardiaque ainsi qu'une diminution de la résistance vasculaire et de la pression capillaire pulmonaires.[57, 58] Ces effets peuvent être observés dans les 5 à 15 min suite à une administration intraveineuse ciblant les concentrations plasmatiques situées à l'intérieur de la marge thérapeutique (100-300 ng/ml).[59]

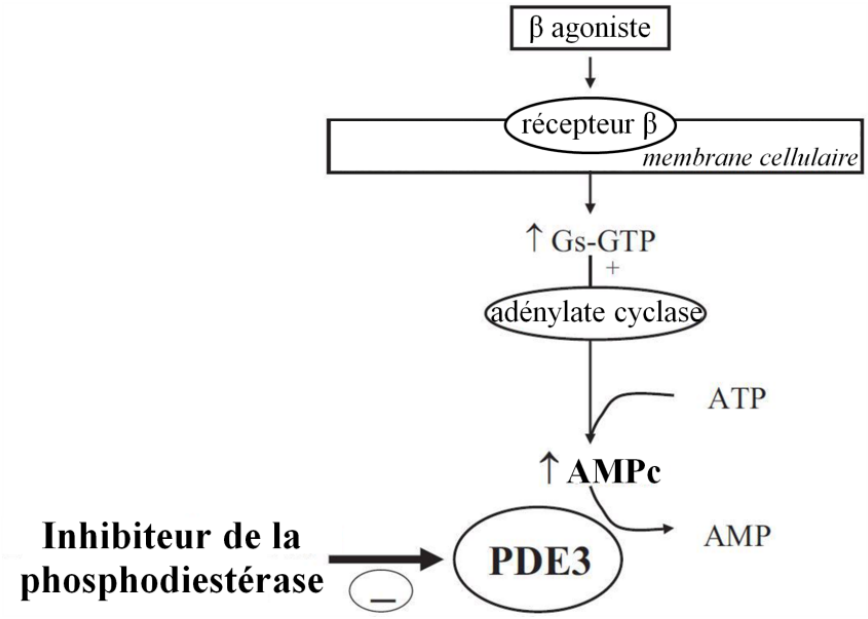


**Figure 7**. Mécanisme d'action des inhibiteurs de la phosphodiestérase 3 (PDE3). Gs, protéine G; GTP, guanosine triphosphate; ATP, adénosine triphosphate; AMPc, adénosine monophosphate cyclique; AMP, adénosine monophosphate. Adaptée de Overgaard.[60]

## 3.2. Utilisations cliniques

### 3.2.1. Voie intraveineuse

La milrinone a été démontrée efficace pour faciliter le sevrage de la CEC.[55] Toutefois, son administration intraveineuse est associée à plusieurs effets secondaires, notamment une incidence élevée d'hypotension systémique qui représente la situation la plus inquiétante.[61, 62] Ainsi, l'administration de la milrinone intraveineuse pour prévenir un sevrage de CEC difficile n'est pas sans risque, provoquant potentiellement une augmentation non nécessaire de la consommation myocardique en oxygène, une altération de la fonction plaquettaire et une hypotension systémique sévère.[63] Chez certains patients plus à risque, l'augmentation de la force contractile cardiaque engendrée par la milrinone intraveineuse peut causer une obstruction de la chambre de chasse des ventricules gauche et droit qui, d'ailleurs, représente une contre-indication à l'utilisation des agents inotropes.[64] Par conséquent, toutes ces complications entraînent le besoin d'un support pharmacologique supplémentaire pour sevrer le patient de la CEC.[65, 66]

Enfin, un sevrage difficile de la CEC serait d'autant plus évité par l'administration d'agents pharmacologiques ayant la capacité d'augmenter la pression de perfusion coronarienne et réduire les pressions artérielles pulmonaires (i.e. la post-charge ventriculaire droite) sans toutefois affecter les pressions artérielles systémiques, ni augmenter la contractilité myocardique en situation d'obstruction dynamique ventriculaire. Or, l'administration de la milrinone par inhalation représente une alternative avantageuse permettant à la fois de cibler le système vasculaire pulmonaire et minimiser l'exposition systémique pour éviter l'hypotension sévère généralement associée à son administration intraveineuse.

### 3.2.2. Voie pulmonaire (inhalation)

Dans un contexte de chirurgie cardiaque chez des patients adultes, la milrinone administrée par inhalation a été démontrée efficace pour le traitement de l'HP, et ce, sans provoquer d'hypotension systémique.[67-69] Dans ces études, lorsque la milrinone est utilisée en tant que traitement pour l'HP, une diminution des résistances vasculaires pulmonaires (RVP),[67-69] et des pressions artérielles pulmonaires (PAP)[68, 69] a été observée.

Lorsque la milrinone inhalée est administrée en prophylaxie (pré-CEC) chez des patients jugés à risque (HP pré-CEC) devant subir une chirurgie cardiaque, les mêmes effets de diminution des RVP[70, 71] et des PAP[71, 72] ont également été observés sans aucun cas d'hypotension systémique rapporté. De plus, la milrinone inhalée pré-CEC comparativement à post-CEC a été associée à une réduction d'incidence de sortie de CEC difficile.[72] D'autres études ont dosé les niveaux plasmatique de facteurs inflammatoires (IL-6, TNF-α) normalement impliqués lors de l'utilisation de la CEC en chirurgie cardiaque et ont observé que ceux-ci étaient significativement réduits suite à l'administration prophylactique de la milrinone inhalée.[73, 74] Tous ces effets préventifs relié à l'administration de la milrinone inhalée pré-CEC observés chez les patients en chirurgie cardiaque appuient les résultats précédemment observés dans le même contexte de prévention chez un modèle animal de CEC,[10] i.e., l'absence de dysfonction endothéliale pulmonaire et d'hypotension systémique, ainsi que la prévention du syndrome de reperfusion pulmonaire et d'incidence de sortie de CEC

difficile. L'utilisation clinique de la milrinone administrée par voie intraveineuse en chirurgie cardiaque a été résumée par Denault et al. en 2006.[58] De manière similaire, le Tableau **III** présente l'utilisation clinique de la milrinone inhalée en chirurgie cardiaque, incluant une étude pédiatrique[75] et trois rapports de cas.[76-78] Jusqu'à ce jour, aucune étude pharmacocinétique n'a été publiée suite à l'administration de la milrinone inhalée.

**Tableau III.** Utilisation clinique de la milrinone inhalée (Partie 1).

| Ref. | Author | Date | Population | n | Type | Groups | Dosage | Timing | Type of nebilizer |
|---|---|---|---|---|---|---|---|---|---|
| [67] | **Haraldsson** | **2001** | Cardiac surgical patients with post-op PH (mPAP >25 mmHg & PVR >200 dyn·sec·cm$^{-5}$) | 20 | Observational | Part I: iMil (3 doses)<br><br>Part II: iPGI2 vs. iPGI2 + iMil | Part I: 3 mg iMil (3 incremental diluted doses: 0.25 mg/ml, 0.5 mg/ml, 1mg/ml) over 30 min (3 subsequent 10 min periods)<br><br>Part II: $iPGI_2$ (10 ug/ml) over 10 min + ($iPGI_2$ (10 ug/ml) + iMil (1 mg/ml)) over 10 min | post-op (ICU) | Jet |
| [68] | **Sablotzki** | **2005** | Heart transplant candidates with/without PH (mPAP >30mmHg) | 18 | Observational | PH vs. no PH | 2 mg iMil (diluted in 3 ml) within 15min (25-50 ug/ml) | per-diagnostic (right heart catheterization) | Ultrasonic |
| [72] | **Lamarche** | **2007** | Cardiac surgical patients under CPB with PH | 70 | Retrospective | Pre- vs. Post-CPB | 5 mg iMil (1 mg/ml) over 5 min (50-80 ug/kg) | pre-/post-CPB | Jet |
| [76] | **Buckley** | **2007** | Patient with acute PH | 1 | Case report | IV treprostinil + iNO + iMil | iNO 20 ppm + treprostinil 2 ng/kg/min + iMil (diluted 0.5 mg/ml) at rate 2 L/min (4mg/h) for 3 days<br><br>iMil decreased to 1 mg/h over 24 h on day 4 & tapered off until discontinuation on day 9. | ICU (added salvage therapy) | Jet |
| [69] | **Wang** | **2009** | Cardiac surgical patients under CPB with PH (mPAP >25mmHg) & post-op PH (mPAP >25 mmHg & PVR >200 dyn·sec·cm$^{-5}$) | 48 | Randomized Controlled Trial | iMil vs. IV Mil | 24 mg iMil (1 mg/ml) over 4 h<br><br>IV Mil bolus 50 ug/ml + infusion 0.5 ug/kg/min for 4 h | post-op (ICU) | Jet |
| [70] | **Hegazy** | **2010** | Cardiac surgical patients with PH (sPAP >30 mmHg or mPAP >25 mmHg) | 92 | Randomized Controlled Trial | iMil vs. $iPGI_2$ | 5 mg iMil (1 mg/ml) over 5 min (50-80 ug/kg)<br><br>5 ml $iPGI_2$ (15 ug/ml) over 5 min | pre-CPB | Jet |

| Ref. | Author | Date | Population | n | Type | Groups | Dosage | Timing | Type of nebilizer |
|---|---|---|---|---|---|---|---|---|---|
| [75] | **Singh** | **2010** | Cardiac surgical patients (children <12yr) with PH (mPAP >30 mmHg) | 35 | Randomized Controlled Trial | iMil vs. iNTG | iMil or iNTG (diluted in 3ml) over 10 min (50 µg/kg) after pulmonary vasodilatation by 100% oxygen for 10 min | pre-CPB (pre-anesthesia) | Jet |
| [77] | **Carev** | **2010** | Cardiac surgical patients with severe PH (sPAP 80-90 mmHg & mPAP 40-50+ mmHg) | 2 | Case report | IV Mil + iMil | IV Mil 0.5-0.75 ug/kg/min<br>+ NTG 1-3 ug/kg/min<br>+ iMil (diluted 0.5 mg/ml) at rate 0.3 ml/min over 70 min | post-op (ICU) | Jet |
| [73] | **Guo** | **2011** | Cardiac surgical patients under CPB | 30 | Randomized Controlled Trial | iMil vs. placebo | 5 mg iMil (diluted in 5 ml) per 8 h two days pre-op | pre-op | NA |
| [74] | **Gong** | **2012** | Cardiac surgical patients under CPB | 30 | Randomized Controlled Trial | iMil vs. placebo | 15 ml iMil (0.1%) over 15 min | pre-CPB (pre-anesthesia) | Ultrasonic |
| [71] | **Denault** | **2014** | Cardiac surgical patients with PH (sPAP >30 mmHg or mPAP >25 mmHg) | 21 | Randomized Controlled Trial | iMil vs. placebo | 5 mg iMil (1 mg/ml) over 5 min (50-80 ug/kg). | pre-CPB | Jet |
| [78] | **St-Pierre** | **2014** | Cardiac surgical patients with PH (mPAP 74 mmHg) | 1 | Case report | iPGI2 + iMil | 75 ug $iPGI_2$<br>+ 5 mg iMil (1 mg/ml) over 5 min (50-80 ug/kg) | pre-CPB | Jet |

**Tableau III.** Utilisation clinique de la mirinone inhalée (Partie 2).

| Ref. | Author | Data collection | Results |
|---|---|---|---|
| [67] | **Haraldsson** | Bsl, after each 10 min periods & 20 min after end inhalation. | iMil (1 mg/ml) ↓mPAP (6%), ↓PVR (20%), ↓TPG (15%), ↓PVR/SVR (17%) & all values returned to bsl 20 min after end inhalation.<br>$iPGI_2$ had similar vasodilator effects but iMil + $iPGI_2$ potentiated (PVR, SV) & prolonged >20 min (mPAP, PVR, TPG).<br>No systemic hypotension in all groups. |
| [68] | **Sablotzki** | Bsl, 10, 30, 60 min after start inhlation. | PH group: ↓mPAP, ↓PVRI (25%), ↓TPG after 10 min & ↓PCWP, ↑RVEF after 30 min. mPAP & PVRI values returned to bsl after 30 min. In non-PH group ↑PCWP.<br>No systemic hypotension in all groups. |
| [72] | **Lamarche** | After CPB | iMil pre-CPB ↓mPAP, ↓CPB reinitiation, ↓DSB, ↑LV FAC.<br>iMil post-CPB ↑mPAP, ↓mAP/mPAP ratio.<br>Both groups ↓mAP, ↑HR, ↑CI, ↓$PaO_2/FiO_2$.<br>No systemic hypotension in all groups.<br>CPB time, cross clamp time & iMil pre-CPB identified as potential DSB risk factor. |
| [76] | **Buckley** | Every hour on day 1 & daily thereafter until day 9. | iMil ↑$SvO_2$ (12%) within 10 min starting inhalation.<br>No systemic hypotension or HR effect. |
| [69] | **Wang** | 0, 1, 2, 3, 4 h after start inhalation & 15, 30, 45, 60 min after end inhalation. | iMil ↑$PaO_2/FiO_2$, ↓Qs/Qt. IV Mil ↓mAP & ↓SVR after 2-4h (induced systemic hypotension; n = 2).<br>Both groups ↓mPAP, ↓PVR & ↑CI.<br>mPAP & PVR values returned to bsl after 60 min end inhalation. |
| [70] | **Hegazy** | 0, 15 min after end inhalation & 15, 30 min post-CPB weaning & at arrival to ICU. | Both groups ↓PVR, ↓mPAP, ↑CI, ↑HR & ↑RVEF post-CPB & at arrival to ICU.<br>No systemic hypotension in all groups. |
| [75] | **Singh** | | Both groups ↓sPAP, ↓dPAP, ↓mPAP, ↓PVRI, ↓SVRI & ↑Qp/Qs.<br>No systemic hypotension in all groups. |
| [77] | **Carev** | Bsl & 0, 12, 24 h after end inhalation. | ↓PVR (40-43%), ↓mPAP (6-8%) after inhalation & ↓PVR (32-39%), ↓mPAP (2-25%), ↑mAP/mPAP (16-41%) 24 h after inhalation.<br>No systemic hypotension. |
| [73] | **Guo** | 0, 30 min post-aortic unclamping & 0, 24, 72, 168 h post-op. | Both groups ↑ all factors plasma levels post-CPB.<br>iMil group PVR, OI, TNF-alpha, IL-6, MDA & venous/arterial ratio leucocyte count were lower post-op. |
| [74] | **Gong** | 0 min, imm. pre-CPB & 0, 24 h post-op. | Both groups ↑ all factors plasma levels post-CPB & 24 h post-op.<br>iMil group Il-6, TNF-alpha, MMP-9 were lower at the end of surgery. |
| [71] | **Denault** | 0 min, end inhalation, 20 min after end inhalation (pre-CPB), post-CPB & at chest closure. | iMil ↓PVR.<br>In control group ↑ right-sided cavity dimensions (↑RVEDA, ↑RADt & ↑TAPSE).<br>No systemic hypotension in all groups. |
| [78] | **St-Pierre** | Bsl, during, after (pre-CPB) inhalation & post-CPB. | ↓PVR (9-40%), ↓mPAP (12-41%) during & after inhalation & post-CPB (max effect).<br>No systemic hypotension. |

Bsl, baseline; CPB, cardiopulmonary bypass; CI, cardiac index; dPAP, diastolic pulmonary pressure; DSB, difficult separation from bypass; $FiO_2$, fraction of inspired $O_2$; HR, heart rate; ICU, intensive care unit; IL-6, Interleukin-6; iMil, inhaled milrinone; iNO, inhaled nitric oxide; iNTG, inhaled nitroglycerin; $iPGI_2$, inhaled prostacyclin; LVFAC, left ventricular fractional area change; mAP, mean artery pressure; MDA, malondialdehyde; MMP-9, metaloproteinase-9; mPAP, mean pulmonary artery pressure; OI, oxygenation index; $PaO_2$, arterial $O_2$ tension; PCWP, pulmonary capillary wedge pressure; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; PVRI, pulmonary vascular resistance index; Qs/Qt, intrapulmonary shunt fraction; RADt, tranverse dimensions of the right atria; RVEDA, right ventricular end diastolic area; RVEF, right ventricular ejection fraction; sPAP, systolic pulmonary artery pressure; SV, stroke volume; $SvO_2$, venous oxygen saturation ; SVR, systemic vascular resistance; SVRI, systemic vascular resistance index; TAPSE, triscupid systolic annular plane excursion; TPG, transpulmonary gradient.

# CHAPITRE 4. Pharmacocinétique des médicaments

La pharmacologie clinique est la discipline scientifique qui étudie le devenir et l'effet des médicaments chez l'humain. Elle est subdivisée en deux sous-disciplines scientifiques, soit la pharmacocinétique et la pharmacodynamie, et consiste à décrire la relation entre la dose administrée et l'effet biologique observé (désirable ou indésirable) (Figure **8**).

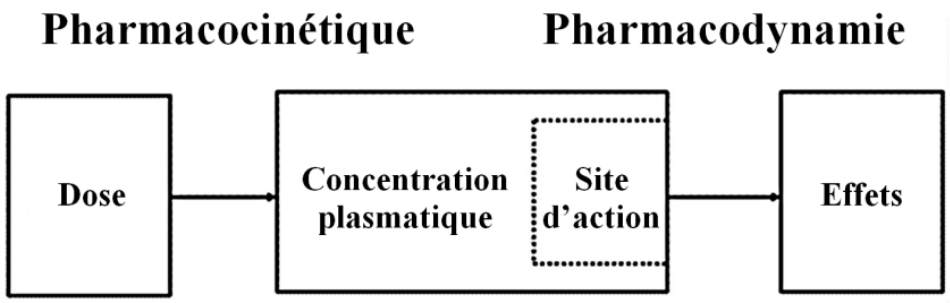


**Figure 8**. Les deux sous-disciplines scientifiques de la pharmacologie clinique: la pharmacocinétique et la pharmacodynamie.

La pharmacocinétique (PK) étudie le devenir du médicament suite à son administration dans l'organisme, en d'autres mots « ce que fait l'organisme au médicament ». Elle décrit la relation entre la dose administrée et les concentrations du médicament et s'intéresse à l'évolution temporelle des concentrations du médicament dans l'organisme suite aux mécanismes d'absorption, de distribution, du métabolisme et d'élimination (ADME). Les propriétés physico-chimiques de chaque médicament, i.e., poids moléculaire (PM), coefficient de partage octanol/eau (Log P), propriétés acido-basiques (pKa), lipophilicité, hydrosolubilité et autres, exercent un impact direct sur leur comportement à chacune des étapes pharmacocinétiques.

## 4.1. Absorption

Les voies d'administration pour les médicaments comprennent notamment les voies: intravasculaire (intraveineuse), orale, pulmonaire (inhalée), rectale, intramusculaire, sous-cutanée, cutanée, oculaire, buccale et perlinguale. Lorsque le médicament est administré par voie extravasculaire, celui-ci doit confronter diverses barrières et

obstacles limitant sa capacité à rejoindre la circulation systémique. L'absorption est le processus par lequel le médicament quitte son site d'administration et traverse les membranes biologiques pour pénétrer dans la circulation sanguine. Plusieurs facteurs peuvent influencer le passage transmembranaire du médicament au site d'absorption, incluant les aspects physiologiques de la membrane et les propriétés physico-chimiques du médicament.

Le paramètre de biodisponibilité ($F$) est utilisé pour caractériser l'absorption d'une formulation pharmaceutique spécifique d'un médicament. Ce terme a été défini comme étant la fraction de la dose administrée du médicament qui atteint la circulation systémique.[79] Généralement, la biodisponibilité est exprimée par rapport à la voie intraveineuse, considérée comme référence avec absorption totale ($F$=1). On parle alors de biodisponibilité absolue. Lorsqu'une voie extravasculaire est utilisée comme référence pour l'estimation de la biodisponibilité, on parle de biodisponibilité relative ($F$=1-$E$). Le coefficient d'extraction ($E$) représente l'extraction séquentielle au niveau des différents organes.[80]

$$E = E_{Gastrique} \times E_{Hépatique} \times E_{Rénale} \times E_{...}$$

L'effet de premier passage pré-systémique (intestinal, hépatique, pulmonaire ou autre) est l'ensemble des phénomènes modifiant la biodisponibilité du médicament, i.e. dissolution, dégradation, diffusion, transport actif et métabolisme. La présence de protéines membranaires, agissant comme transporteurs actifs, peut contribuer à l'absorption du médicament (influx) ou protéger l'organisme contre celui-ci (efflux). Dans le cas d'une administration extravasculaire, l'observation de la concentration maximale ($C_{max}$) et du temps auquel celle-ci apparaît ($t_{max}$) permet d'apprécier subjectivement la vitesse d'absorption du médicament. Ainsi, pour une même dose du même médicament, la vitesse d'absorption sera d'autant plus lente si le $C_{max}$ est faible et le $t_{max}$ décalé dans le temps. En pharmacocinétique, l'aire sous la courbe ($ASC$) des concentrations plasmatiques ($C_p$) en fonction du temps ($t$) est souvent évaluée, puisqu'elle donne un aperçu de l'ampleur de l'exposition systémique totale au médicament et de sa clairance.

## 4.2. Distribution

Une fois absorbé, le médicament est distribué dans les différents tissus de l'organisme. Il s'agit d'un processus généralement réversible qui consiste en un transfert du médicament de la circulation systémique vers les différents tissus selon son affinité pour ceux-ci (aspects physiologiques et propriétés physico-chimiques du médicament). Encore une fois, les facteurs physico-chimiques (PM, pKa, Log P) et physiologiques (composition de la membrane, débit sanguin), incluant la présence de transporteurs membranaires (influx et efflux), jouent un rôle majeur dans la distribution tissulaire du médicament. Au niveau de la circulation sanguine, une partie du médicament peut se lier aux protéines plasmatiques, seule la fraction libre (non liée) traverse les membranes biologiques pour se distribuer vers les autres tissus.

Le volume de distribution apparent ($V_d$) est le paramètre pharmacocinétique utilisé pour décrire l'importance de la distribution du médicament dans l'organisme, sans nécessairement correspondre à un volume physiologique réel. Ce terme représente la relation de proportionnalité entre la quantité ou la dose intraveineuse ($D_{iv}$) et la concentration de médicament dans l'organisme.[80]

$$V_d = \frac{D_{iv}}{C_p}$$

Étant donné que, chez l'homme, les concentrations du médicament sont souvent mesurées dans le sang (plasma), il est possible de juger de l'importance de la distribution du médicament en mettant en relation la concentration plasmatique (mesurée) à un temps donné et la quantité de médicament (connue) dans le corps au même temps

## 4.3. Métabolisme et élimination

Dans l'organisme, le médicament est éliminé de manière irréversible sous forme inchangée et/ou biotransformée par des processus d'excrétion et/ou de métabolisme, respectivement. Le foie constitue le site principal de biotransformation où se forment les métabolites qu'ils soient actifs ou inactifs. Le rein représente le principal organe d'excrétion du médicament sous forme inchangée et de ses métabolites plus

hydrosolubles et généralement inactifs. Certains médicaments ou leurs métabolites sont également excrétés dans la bile.

Le métabolisme, ou biotransformation, constitue le mécanisme d'élimination de la plupart des médicaments et consiste à faire subir une modification irréversible de la structure chimique du médicament via l'intervention d'un système enzymatique endogène. Les réactions de biotransformation sont réparties en quatre catégories selon leur nature chimique: oxydation, réduction, hydrolyse et conjugaison. Souvent, un médicament est biotransformé par une réaction d'oxydation, de réduction ou d'hydrolyse, dite de phase I, suivie d'une réaction de conjugaison, dite de phase II, du métabolite formé. Les réactions de phase I sont principalement catalysées par les enzymes de la superfamille des cytochromes P-450 et consistent souvent en l'introduction d'un groupement fonctionnel ou réactif. Les réactions de phase II sont catalysées par les enzymes de synthèse (glucuronosyltransférase, glutathion-S-transférase et N-acétyltransférase sulfotransférase) et consistent en une conjugaison avec l'acide glucuronique, le glutathion ou un sulfate, respectivement. Les réactions de biotransformation peuvent avoir comme résultat une inactivation, activation ou potentialisation de l'effet du médicament originalement administré. Le métabolite formé par réaction d'inactivation est généralement plus polaire et hydrosoluble et ce, afin de favoriser son élimination par les voies rénales et biliaires.

L'excrétion d'un médicament dans l'urine résulte des processus de filtration glomérulaire, sécrétion tubulaire et réabsorption tubulaire. La filtration glomérulaire consiste au passage du médicament à travers la paroi du glomérule selon un gradient de pression favorable. La membrane de la paroi glomérulaire agit comme un tamis ne laissant passer que les petites molécules (<20 000 Da). Puisque les macromolécules, e.g. protéines plasmatiques, ne peuvent pas traverser la membrane, seule la fraction libre du médicament peut être filtrée et excrétée. La vitesse de filtration glomérulaire (extraction rénale), étant également fonction du débit sanguin rénal, se voit diminuée en présence d'insuffisance cardiaque. La sécrétion tubulaire permet le passage du médicament du sang vers la lumière tubulaire par un mécanisme de transport actif (OAT ou OATP pour acides faibles et OCT pour bases faibles). Comme tout système de transporteurs, la

sécrétion tubulaire est saturable et sujette à une inhibition compétitive. Bien que seule la fraction libre du médicament puisse être sécrétée, le complexe médicament-protéine peut se dissocier au site de sécrétion tubulaire selon son degré d'affinité pour la protéine plasmatique ou le transporteur. Enfin, la réabsorption tubulaire permet le passage en sens inverse du médicament de l'urine au plasma. Elle se fait généralement par diffusion passive et son importance varie selon les propriétés physico-chimiques (PM, pKa, Log P) du médicament. La réabsorption tubulaire de l'eau se fait passivement, ayant pour effet la concentration des urines. Le degré de réabsorption tubulaire passive est fonction inverse du débit urinaire et dépend également du pH tubulaire. Pour plusieurs substances endogènes (électrolytes, glucose, acides aminés et vitamines), la réabsorption tubulaire se fait activement. La clairance rénale ($Cl_R$) est la résultante des contributions relatives des trois processus rénaux.[80]

$$Cl_R = Filtration\ glomérulaire + Sécrétion - Réabsorption$$

L'élimination se caractérise par la diminution des concentrations du médicament dans la circulation systémique. Trois paramètres pharmacocinétiques peuvent servir à évaluer l'élimination d'un médicament. La clairance systémique ou totale ($Cl$) est la mesure de l'efficacité de l'organisme à épurer un volume de sang du médicament par unité de temps et résulte de l'ensemble des clairances des différents organes impliqués dans le processus d'élimination.[80]

$$Cl = Cl_R + Cl_{Hépatique} + Cl_{Pulmonaire} + Cl_{...}$$

La constante de vitesse d'élimination ($k_e$) est la mesure de la vitesse à laquelle une fraction de la quantité de médicament est éliminée de l'organisme. Cette constante varie selon deux paramètres indépendants, soit la clairance totale et le volume de distribution apparent.[80]

$$Cl = k_e \times V_d$$

Le temps de demi-vie d'élimination apparente ($t_{1/2}$) découle directement de la constante de vitesse d'élimination et représente le temps nécessaire pour réduire de moitié la quantité de médicament dans l'organisme.[80]

$$k_e = \frac{\ln(2)}{t_{1/2}}$$

## 4.4. Pharmacocinétique de la milrinone

Suite à une administration orale (1-12 mg) chez des volontaires sains, la milrinone est rapidement et presqu'entièrement absorbée ($F$=0.92).[81] Il est démontré (par ultracentrifugation) que la milrinone est liée à 70-80% aux protéines plasmatiques dans l'intervalle de concentrations plasmatiques de 70-400 ng/ml.[81] La mirinone est principalement excrétée dans l'urine, sous forme inchangée (83%) ou conjuguée O-glucuronide (12%).[81, 82] Des quantités beaucoup plus faibles peuvent également être retrouvées dans les selles (<5%). Chez les volontaires sains, l'élimination de la milrinone est rapide avec environ 60% de la dose retrouvée dans l'urine durant les deux premières heures suivant l'administration et 90% après huit heures. Les valeurs moyennes de $Cl_R$ de la milrinone sont de 17-24 L/h alors que celles des métabolites sont encore plus élevées, indiquant la présence de sécrétion active.[81, 82] Les valeurs moyennes de $C_{max}$ (162 ng/ml) et de $t_{max}$ (0.6 h) observées chez les sujets sains avec une fonction rénale normale sont augmentées chez les patients atteints d'insuffisance rénale modérée à sévère (210 ng/ml et 1.2 h, respectivement).[82] Selon le degré de gravité de l'insuffisance rénale (modérée-sévère), la valeur moyenne de $t_{1/2}$ (0.9 h) est augmentée à 1.8-3.2 h, respectivement. Chez les patients atteints d'insuffisance cardiaque congestive, des valeurs moyennes similaires (plus élevées) de $C_{max}$ (208-218 ng/ml),[83, 84] $t_{max}$ (1.5-1.7 h)[83-85] et $t_{1/2}$ (2.3 h)[83] ont également été rapportées, et ce, malgré une légère diminution notée au niveau de la biodisponibilité (0.76).[83]

En raison du taux de mortalité élevé associé à l'administration orale,[86] la milrinone est plutôt administrée par voie intraveineuse chez les patients (généralement sous forme d'une dose de départ de 50 ug/kg sur 10 min suivie d'une dose de maintien de 0.375-0.75 ug/kg/min). La marge thérapeutique des concentrations plasmatiques pour la

milrinone est de 100-300 ng/ml[59] et la concentration toxique est au-delà de 300 ng/ml.[85] Suivant une perfusion constante (0.50 ug/kg/min) maintenue pendant 6-12 heures, la concentration plasmatique à l'état d'équilibre ($C_{éq}$) est approximativement de 200 ng/ml.[87, 88]

Suivant l'administration d'une injection intraveineuse (12.5-125 ug/kg) ou d'une perfusion intraveineuse (0.20-0.70 ug/kg/min) chez des patients atteints d'insuffisance cardiaque congestive, la milrinone affiche un $V_d$ de 0.30-0.47 L/kg, une *Cl* de 0.11-0.16 L/h/kg et une $t_{1/2}$ de 1.5-2.6 h.[83, 88-90] Ces paramètres PK ne sont pas affectés par la dose, alors que l'*ASC* est significativement dose-dépendante.[88]

Chez les patients subissant une chirurgie cardiaque sous CEC, les valeurs moyennes de $V_d$ (0.31-0.47 L/kg), *Cl* (0.13-0.22 L/h/kg) et $t_{1/2}$ (1.7-2.6 h) sont comparables à celles observées chez les patients atteints d'insuffisance cardiaque congestive.[91-93] et également indépendants de la dose administrée.[93, 94] Par ailleurs, lorsqu'un modèle à trois compartiments est utilisé pour analyser les données PK, les paramètres diffèrent de ceux rapportés suivant une analyse non compartimentale ou lorsqu'un modèle à deux compartiments est appliqué.[93, 94]

**Tableau IV.** Pharmacocinétique de la mirinone (Partie 1).

| Ref. | Author | Date | Population | n | Dose | Sampling (h) | $T_{max}$ (h) | $C_{max}$ ($C_{ss}$) (ng/ml) |
|---|---|---|---|---|---|---|---|---|
| [81] | **Stroshane** | **1984** | Healthy male volunteers | 21 | 10, 30, 45, 60, 75, 100, 125 µg/kg IV bolus | 24 | | 84 ± 28, 256 ± 39, 431 ± 152, 680 ± 62, 706 ± 299, 559 ± 146, 830 ± 370 |
| | | | Healthy male volunteers | 18 | 1, 2.5, 5, 7.5, 10, 12.5 mg orally | 24 | 1.1 ± 0.6 | 28 ± 12, 41 ± 9, 116 ± 22, 152 ± 30, 158 ± 63, 234 ± 106 |
| [83] | **Stroshane** | **1984** | NYHA III & IV | 6 | 12.5 - 75 µg/kg IV bolus | | | |
| | | | NYHA III & IV | 8 | 0.2-0.7 µg/kg/min IV infusion 18h | | | |
| | | | NYHA III & IV | 10 | 2.5, 5, 10 mg orally | | 1.7 ± 1.5 | D2: 218 ± 130 |
| [89] | **Wilson** | **1984** | NYHA II & III | 11 | 12.5 - 75 µg/kg IV bolus | | | |
| [90] | **Benotti** | **1985** | NYHA III & IV | 13 | 12.5, 25, 50, 75 µg/kg IV bolus | 6 | | 81 ± 40, 169 ± 32, 272 ± 50, 454 ± 55 |
| [85] | **Kubo** | **1985** | NYHA III & IV | 34 | 2.5, 5, 7.5, 10, 12.5, 15 mg orally | 6 | 1.5 | >300 |
| [88] | **Edelson** | **1986** | NYHA III & IV | 26 | 12.5, 25, 50, 75, 100, 125 µg/kg IV bolus | 6 | | |
| | | | | | 0.2, 0.45, 0.7 µg/kg/min IV infusion 18h | 26 | | (96 ± 10, 200 ± 30, 255 ± 17) |
| | | | NYHA III & IV | 21 | 2.5, 5, 7.5, 10, 12.5, 15 mg orally | 6 | | 74 ± 7, 134 ± 11, 221 ± 13, 307 ± 17, 306 ± 32, 446 ± 50 |
| [82] | **Larsson** | **1986** | Healthy volunteers | 7 | 5 mg orally | 24 | 0.6 ± 0.4 | 162 ± 42 |
| | | | Renal impaired patients (CRI I) | 7 | 5 mg orally | 24 | 1.2 ± 1.0 | 210 ± 80 |
| | | | Renal impaired patients (CRI II) | 7 | 5 mg orally | 24 | 1.2 ± 0.5 | 210 ± 59 |
| [87] | **Anderson** | **1986** | NYHA III & IV | 189 | 50 µg/kg IV bolus over 10 min + 0.50 µg/kg/min | 48 | | (D1: 370 ± 32, 215 ± 11, D2: 179±10) |
| [84] | **Nanimatsu** | **1993** | NYHA II - IV | 11 | 7.5 mg orally | 24 | 1.5 ± 0.4 | 208 ± 22 |
| [91] | **Das** | **1994** | Cardiac surgical patients with CPB | 7 | 50 µg/kg IV bolus over 10 min + 0.50 µg/kg/min | 6 | | ~250 - 450, (262 ± 85) |
| [94] | **Bailey** | **1994** | Cardiac surgical patients with CPB | 25 | 25, 50, 75 µg/kg IV bolus pre-end CPB, 50 µg/kg + 0.50 µg/kg/min pre-end CPB, 50 µg/kg IV bolus after CPB | 16 | | >150: ~200, 600, 750 |
| [92] | **De Hert** | **1995** | Cardiac surgical patients with CPB | 20 | 20, 40 µg/kg IV bolus over 15 min + 0.50 µg/kg/min pre-end CPB | 4 | | ~180, 260 |
| [93] | **Butterworth** | **1995** | Cardiac surgical patients with CPB | 29 | 25, 50 and 75 µg/kg IV bolus over 1 min | 6 | | 460, 610, 1170 |

**Tableau IV.** Pharmacocinétique de la milrinone (Partie 2).

| Ref. | Author | Population | PK analysis | Cl (L/h/kg) | $V_d$ (L/kg) | $T_{1/2}$ (h) | $Cl_R$ (L/h) | F | Dose recovered from urine (%) |
|---|---|---|---|---|---|---|---|---|---|
| [81] | **Stroshane** | Healthy male volunteers | 2 cpt. | | 0.32 ± 0.08 | 0.8 | | | |
| | | | NCA | 0.36 ± 0.08 | 0.25 ± 0.06 | | 21.1 ± 2.9 | | 85 ± 10 |
| | | Healthy male volunteers | NCA | 0.41 ± 0.09 | | 0.9 | 23.8 ± 6.4 | 0.92 | 80 ± 11 |
| [83] | **Stroshane** | NYHA III & IV | | 0.11 ± 0.01 | 0.33 ± 0.03 | 2.1 | | | |
| | | NYHA III & IV | | 0.16 ± 0.02 | 0.47 ± 0.05 | 2.0 | | | |
| | | NYHA III & IV | | 0.16 ± 0.01 | 0.52 ± 0.05 | 2.3 | | 0.76 | |
| [89] | **Wilson** | NYHA II & III | | 0.15 ± 0.06 | 0.30 ± 0.12 | 1.5 ± 0.6 | | | |
| [90] | **Benotti** | NYHA III & IV | 2 cpt. | 0.15 ± 0.03 | 0.35 ± 0.02 | 1.7 ± 0.3 | | | |
| [85] | **Kubo** | NYHA III & IV | | | | | | | |
| [88] | **Edelson** | NYHA III & IV | 2 cpt. | 0.11 ± 0.01 | 0.33 ± 0.01 | 2.3 ± 0.1 | | | |
| | | | NCA | 0.13 ± 0.01 | 0.38 ± 0.01 | 2.3 ± 0.1 | | | |
| | | NYHA III & IV | NCA | 0.14 ± 0.01 | 0.47 ± 0.03 | 2.6 ± 0.2 | | | |
| | | | NCA | 0.16 ± 0.000005 | 0.56 ± 0.02 | 2.7 ± 0.1 | | | |
| [82] | **Larsson** | Healthy volunteers | NCA | | | 0.9 ± 0.1 | 17.3 ± 4.7 | | 83 ± 7 |
| | | Renal impaired patients (CRI I) | | | | 1.8 ± 0.7 | 4.9 ± 1.7 | | 59 ± 12 |
| | | Renal impaired patients (CRI II) | | | | 3.2 ± 0.7 | 1.9 ± 0.8 | | 41 ± 19 |
| [87] | **Anderson** | NYHA III & IV | N/A | | | | | | |
| [84] | **Nanimatsu** | NYHA II - IV | N/A | | | | | | |
| [91] | **Das** | Cardiac surgical patients with CPB | NCA | 0.13 ± 0.04 | 0.31 ± 0.13 | 1.7 ± 0.2 | | | |
| [94] | **Bailey** | Cardiac surgical patients with CPB | 3 cpt. | 0.11 | 1.69 | 14.4 | | | |
| [92] | **De Hert** | Cardiac surgical patients with CPB | 1 cpt. | 0.19 ± 0.04, 0.13 ± 0.02 | | 2.1 ± 0.4, 2.6 ± 0.3 | | | |
| [93] | **Butterworth** | Cardiac surgical patients with CPB | 2 cpt. | 0.22 | 0.47 | 1.7 | | | |
| | | | 3 cpt. | 0.07 | 1.08 | 11.6 | | | |

$C_{max}$, maximum concentration; $C_{ss}$, concentration at steady state; Cl, clearance; $Cl_R$, renal clearance; CPB, cardiopulmonary bypass; CRI, chronic renal insufficiency; D, dose; F, bioavailability; NCA, non-compartmental analysis; NYHA, New York Heart Association; PK, pharmacokinetic; $T_{1/2}$, half-life; $T_{max}$: time of maximum concentration; $V_d$, volume of distribution.

# CHAPITRE 5. Administration de médicaments par voie pulmonaire (inhalation)

L'utilisation de médicaments administrés par voie pulmonaire a vraisemblablement débuté dans les années 1950, avec l'arrivée des thérapies pour le traitement de l'asthme. Suite à d'énormes progrès dans le domaine de l'inhalation, incluant la révolution des dispositifs d'inhalation, cette voie d'administration est aujourd'hui très recherchée pour le traitement de nombreuses maladies pulmonaires. Qu'il s'agisse de cibler une administration topique ou systémique, l'inhalation représente une voie d'administration non invasive permettant aux médicaments de se rendre au site d'absorption sans avoir recours à l'injection. La voie pulmonaire peut présenter plusieurs avantages par rapport à d'autres voies d'administration extravasculaires, e.g., une absorption rapide (quelques secondes ou minutes), un faible métabolisme et, par conséquent, une biodisponibilité systémique élevée dans la majorité des cas.

Deux types de dispositifs d'inhalation sont utilisés pour administrer les médicaments par voie inhalée chez les patients soumis à la ventilation mécanique: les inhalateurs-doseurs pressurisés « pressurized metered-dose inhalers » (pMDIs) et les nébuliseurs. Les pMDIs sont les dispositifs d'inhalation les plus utilisés en thérapie par inhalation à travers le monde. Toutefois, très peu de médicaments sont disponibles sous forme de pMDIs et par conséquent, ces derniers sont principalement utilisés pour l'administration de bronchodilatateurs et corticostéroïdes chez les patients souffrant d'obstruction des voies respiratoires.[95-97] Les nébuliseurs produisent des effets thérapeutiques similaires aux pMDIs chez les patients soumis à la ventilation mécanique[95, 98-101] et peuvent être utilisés pour administrer une variété de médicaments ou de formulations incompatibles avec les pMDIs. Le processus de base pour le fonctionnement des nébuliseurs repose sur la conversion d'une solution contenant le médicament dissout en de fines gouttelettes (ou particules liquides) d'aérosol respirables (1-5 μm) jusqu'aux voies respiratoires inférieures.[102]

## 5.1. Efficacité de l'administration

L'efficacité de l'administration de médicaments par voie inhalée sous forme d'aérosol dépend de plusieurs facteurs propres à chaque type de dispositif d'inhalation et du contexte clinique dans lequel il est utilisé.[103] L'ensemble de ces facteurs influence le rendement du dispositif en modifiant le degré de perte de médicament à l'intérieur du circuit, ainsi que le degré et la répartition du dépôt pulmonaire. Par le fait même, les études *in vitro* consistant à évaluer le rendement des dispositifs d'inhalation fournissent des informations importantes lors des études pharmacocinétiques portant sur les médicaments inhalés.

### 5.1.1. Rendement des dispositifs d'inhalation

En 1991, Smaldone[104] a introduit le concept de la dose inhalée qui aujourd'hui est devenu un critère reconnu pour évaluer le rendement des nébuliseurs. Parmi les autres critères d'évaluation se trouvent la dose émise, la dose résiduelle (ou volume résiduel), le temps de traitement, la fraction respirable et la dose inhalée respirable. La dose nominale correspond à la quantité totale de médicament initialement déposée dans le nébuliseur et sert souvent de valeur de référence par rapport à laquelle sont exprimées les autres doses évaluées. La dose émise est définie comme étant la quantité de médicament qui quitte le dispositif d'inhalation et peut également servir de valeur de référence. Le temps de traitement est la durée nécessaire pour compléter la nébulisation, alors que la dose résiduelle correspond à la quantité de médicament restant dans le nébuliseur une fois la nébulisation terminée. La dose inhalée est définie comme étant la quantité de médicament qui est inhalée par le patient. Cette dose inhalée est généralement déterminée dans les conditions cliniques afin d'être la plus représentative possible de la dose disponible pour le patient suite à la nébulisation. Le reste de la dose qui n'est pas inhalé est soit expiré ou perdu par impaction et sédimentation dans le circuit (e.g., tube en T du nébuliseur, connecteur en Y et tube endotrachéal). La fraction respirable est équivalente à la fraction de la dose émise constituée de particules avec un diamètre aérodynamique médian en masse (MMAD) compris entre 1 et 5 μm. Cette caractéristique physique des particules d'aérosol peut être déterminée *in vitro* à l'aide

d'un impacteur à cascade et indique leur potentiel à pénétrer au-delà des voies respiratoires supérieures pour se déposer dans les régions trachéo-bronchique et pulmonaire périphérique.[105] Finalement, la dose inhalée respirable représente une estimation de la dose inhalée vraisemblablement déposée dans les poumons.[106] Cette dernière peut être calculée en multipliant la dose inhalée par la fraction respirable.

Dose inhalée respirable = Dose inhalée X Fraction respirable

### 5.1.2. Diamètre aérodynamique médian en masse

Les particules d'aérosol émises par le dispositif d'inhalation ayant des propriétés optimales ne devraient être ni trop petites (risque d'être expirées), ni trop grosses (risque d'être principalement déposées dans les voies respiratoires supérieures: la bouche et la gorge). Une particule d'aérosol typique (2 μm de diamètre) peut renfermer des centaines de millions de molécules du médicament. En supposant que le nuage d'aérosol généré soit composé de particules d'aérosol ayant des propriétés similaires à celles des gouttelettes d'eau (sphériques et de densité unitaire), la fraction respirable déposée dans les poumons sera fonction du MMAD de ces particules qui est défini pour chaque type de nébuliseur (Figure **9**).[102, 105]

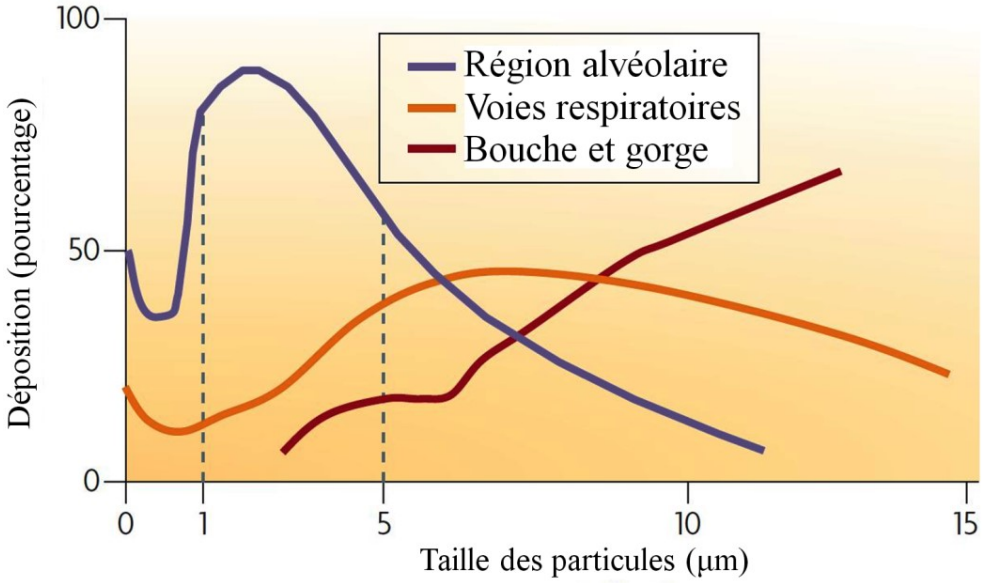


**Figure 9**. Déposition pulmonaire en fonction de la taille des particules. Adaptée de Patton.[107]

Les particules d'aérosol dont le diamètre aérodynamique est d'environ 1–2 μm ont le potentiel d'être déposées en profondeur dans les poumons, et ce, jusqu'à 90% des particules atteignant la région alvéolaire. En ventilation mécanique, il a été démontré que le dépôt pulmonaire profond est obtenu lorsque le MMAD se situe entre 1 et 3 μm,[95] voire inférieur à 2 μm.[108-110] Les particules de plus de 5 μm s'impactent sur la sonde d'intubation, le raccord annelé ou la trachée, alors qu'à l'inverse, les particules inférieures à 0.5 μm diffusent sous l'effet de mouvement Brownien et sont pour la plupart expirées.

## 5.2. Types de nébuliseurs

L'administration efficace de médicaments par voie inhalée peut représenter un défi de taille. Par conséquent, il est primordial, pour celui qui l'administre, de comprendre le fonctionnement des différents types de dispositifs disponibles afin de faire un choix adapté à ses besoins et assurer une utilisation optimale. Il existe trois types de nébuliseurs: 1- les nébuliseurs pneumatiques « jet nebulizers », 2- les nébuliseurs ultrasoniques « ultrasonic nebulizers » et 3- les nébuliseurs à grille perforée ou à tamis vibrant « mesh nebulizers ».[111]

### 5.2.1. Nébuliseurs pneumatiques « jet nebulizers »

Les nébuliseurs pneumatiques utilisent un gaz comprimé (air ou oxygène) pour convertir le liquide en aérosol. Le jet de gaz à haut débit (6-8 L/min) passe à travers une étroite buse Venturi (typiquement 0.3-0.7 mm de diamètre) soit de manière tangentielle ou coaxiale. À sa sortie, le gaz crée une zone de pression négative qui entraîne le liquide dans le réservoir à monter au travers d'un tube capillaire (effet de Bernouilli). L'énergie générée permet de vaincre la tension de surface du liquide qui provoque le cisaillement du liquide en gouttelettes. La majorité de ces gouttelettes sont directement soufflées vers l'extérieur du réservoir, alors qu'une proportion de plus grosses gouttelettes vont plutôt s'impacter sur le bouclier ou les parois pour être recyclées dans le liquide du réservoir (Figure **10**).

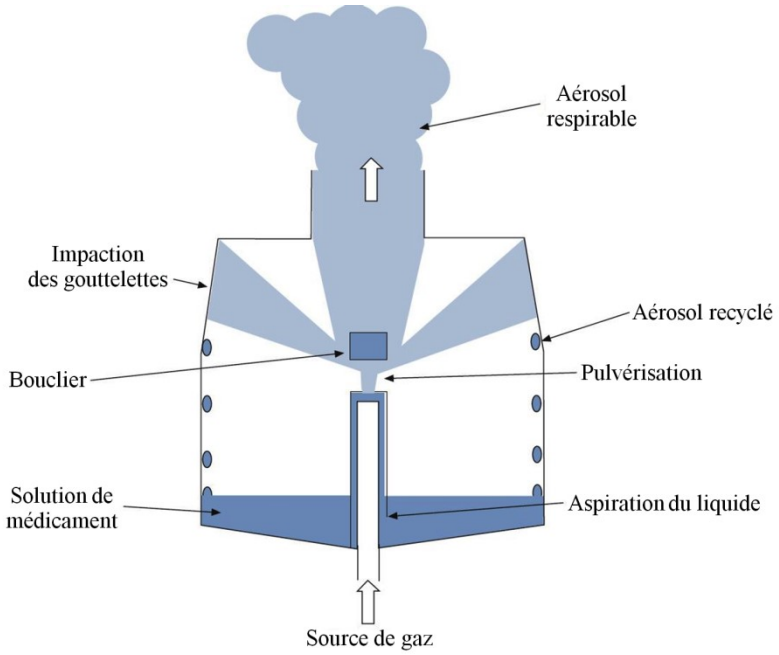


**Figure 10**. Principes de fonctionnement du nébuliseur pneumatique « jet nebulizer ». Adaptée de Vecellio.[112]

L'ajustement du débit de gaz comprimé utilisé pour les nébuliseurs pneumatiques est le déterminant principal de la taille des gouttelettes et du débit de nébulisation. Ainsi, lorsque ce débit passe de 4 à 8 L/min, le MMAD des gouttelettes peut être diminué jusqu'à 50% suivi d'une augmentation linéaire de la proportion de gouttelettes inférieures à 5 µm. L'utilisation des nébuliseurs pneumatiques est très répandue chez les patients sous ventilation mécanique en raison de leur faible coût et la simplicité de leur mode d'utilisation. Par contre, certains désavantages sont également reliés à leur utilisation. Plusieurs études ont démontré leur inefficacité à délivrer les médicaments en quantité suffisante dans les bronches profondes des poumons.[106, 113, 114] D'ailleurs, en plus de nécessiter un branchement de tube supplémentaire au circuit du ventilateur, le débit de gaz externe indispensable à leur fonctionnement modifie les paramètres ventilatoires. En effet, le débit et le volume de gaz générés avec l'utilisation de ces nébuliseurs s'additionnent à ceux programmés au niveau du ventilateur. Par conséquent, cet afflux de gaz supplémentaire peut s'avérer délétère pour certains patients présentant une pathologie pulmonaire en induisant de l'hyperinflation avec risque de barotraumatisme.

### 5.2.2. Nébuliseurs ultrasoniques « ultrasonic nebulizers »

Les nébuliseurs ultrasoniques utilisent un cristal piézo-électrique vibrant à haute fréquence (1-3 MHz) pour convertir le liquide en aérosol. À des intensités ultrasonores suffisamment élevées, le cristal piézo-électrique crée une onde stationnaire dans la solution et fournit l'énergie nécessaire permettant de vaincre la tension de surface du liquide pour produire les gouttelettes. Les grosses gouttelettes sont émises à la crête de l'onde, alors qu'un nuage de plus petites gouttelettes est émis à partir de la partie inférieure (Figure **11**).[115, 116]

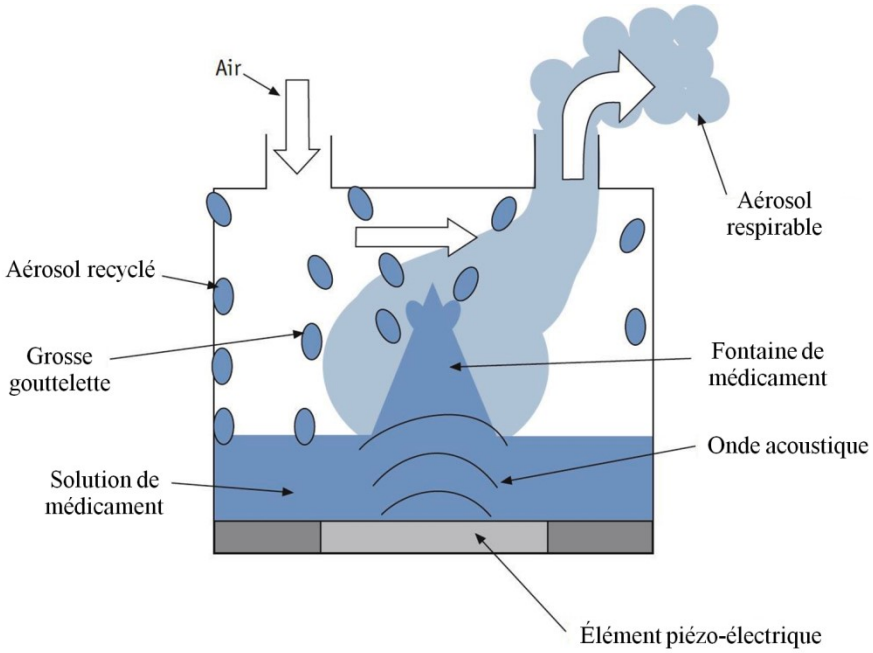


**Figure 11**. Principes de fonctionnement du nébuliseur ultrasonique « ultrasonic nebulizer ». Adaptée de Vecellio.[112]

Avec ce type de nébuliseur, la taille des particules d'aérosol générées est inversement proportionnelle à la fréquence de vibration, tandis que le débit de nébulisation du médicament est directement proportionnel à l'amplitude de l'onde de vibration.[98, 117] Ils ont généralement un débit de nébulisation plus élevé (par conséquent une durée de nébulisation plus courte) et une performance (dose inhalée) supérieure comparativement aux nébuliseurs pneumatiques.[118-120] Cependant, puisque la fréquence est une

caractéristique spécifique à chaque appareil et non réglable, les particules générées sont parfois de diamètre supérieur, associé à un dépôt pulmonaire profond moindre. Une limite importante associée aux nébuliseurs ultrasoniques est l'augmentation de la température à l'intérieur de l'appareil au cours de la nébulisation causant une concentration de la solution médicamenteuse et un risque de dénaturation des composés sensibles à la chaleur. De plus, ces dispositifs ne sont pas adaptés pour la nébulisation de solutions visqueuses qui les rendent très difficiles à nettoyer.

### 5.2.3. Nébuliseurs à grille perforée ou à tamis vibrant « mesh nebulizers »

Les nébuliseurs à grille perforée ou à tamis vibrant font partie d'une nouvelle génération de nébuliseurs conçus pour être compatibles avec la ventilation mécanique.[121] Ils utilisent l'énergie de vibration provenant d'un élément piézo-électrique annulaire situé autour du tamis pour convertir le liquide en aérosol. Les vibrations entraînent la contraction et le relâchement du tamis qui finit par forcer le liquide à passer au travers de ses multiples (jusqu'à 10000) petits orifices coniques de diamètre identique (Figure **12**).[115, 116]

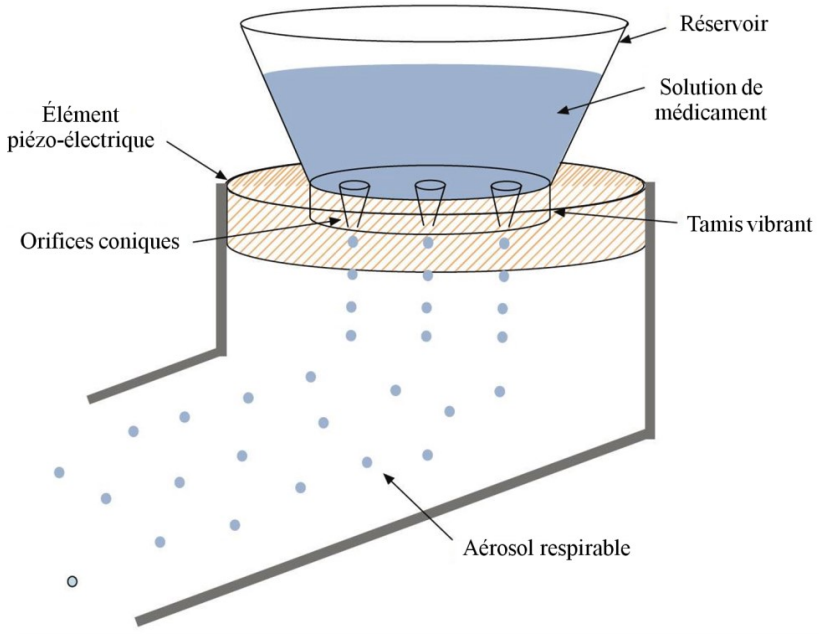


**Figure 12**. Principes de fonctionnement du nébuliseur à grille perforée ou à tamis vibrant « mesh nebulizer ». Adaptée de Vecellio.[112]

La technologie de pointe derrière ces nébuliseurs permet de générer un nuage de gouttelettes de taille plus homogène et une plus grande fraction de particules fines tout en atteignant un débit de nébulisation plus rapide (réduction du temps de traitement).[115, 116, 121-123] De plus, ils ne provoquent pas une augmentation importante de la température de la solution[124-126] et laissent de très petits volumes résiduels comparativement aux nébuliseurs pneumatiques et ultrasoniques. Bien que les nébuliseurs à tamis vibrant soient plus dispendieux, leur taille compacte, leur usage facile et leur fonctionnement silencieux constituent des avantages non négligeables qui en font actuellement le type de dispositif de premier choix pour la nébulisation en ventilation mécanique.[115, 116, 121] Toutefois, les solutions de médicament trop visqueuses peuvent obstruer les pores et altérer leur bon fonctionnement. Malgré le peu d'études cliniques qui ont évalué les nébuliseurs à tamis vibrant lors de leur utilisation chez les patients, plusieurs études *in vitro* ont démontré que leur efficacité à délivrer les particules d'aérosol durant la ventilation mécanique[127] était similaire à celle obtenue avec les nébuliseurs ultrasoniques[128, 129] Par ailleurs, comparativement aux nébuliseurs pneumatiques conventionnels, la déposition pulmonaire mesurée *in vitro* est approximativement de 2 à 3 fois supérieure.[127, 130]

## 5.3. Facteurs déterminants de l'absorption pulmonaire

### 5.3.1. Aspects physiologiques des poumons

Chez l'humain, les voies respiratoires sont divisées en deux catégories: les voies respiratoires supérieures (extra-thoraciques) et les voies respiratoires inférieures (intra-thoraciques). Les voies respiratoires supérieures sont composées du nez, de la bouche, du pharynx et du larynx, alors que les voies respiratoires inférieures sont composées de la trachée, des bronches, des bronchioles et des alvéoles. L'anatomie de la partie intra-thoracique peut être comparée à celle d'un arbre inversé: le tronc et les branches représentent la zone de conduction (trachée, bronches, bronchioles), alors que la partie feuillue équivaut à la zone respiratoire (alvéoles). Les conduits aériens peuvent se ramifier plus de 16 à 17 fois avant d'atteindre la zone respiratoire (Figure **13**).

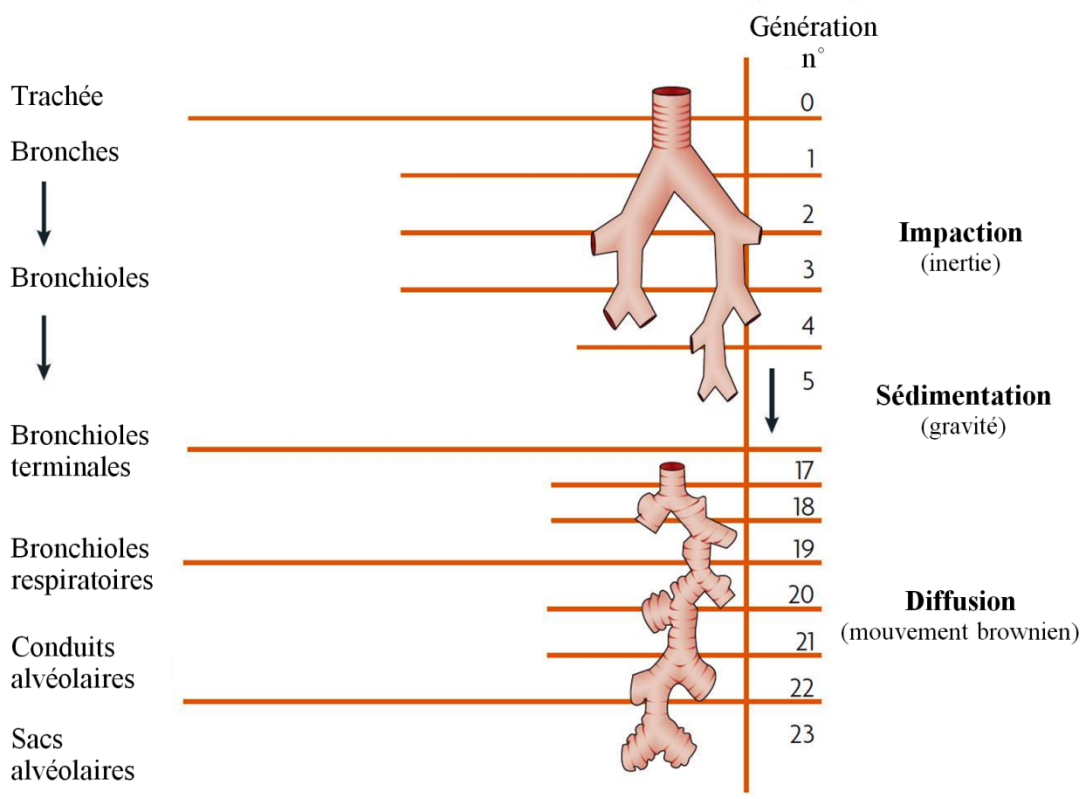


**Figure 13**. Embranchements anatomiques des voies pulmonaires. Adaptée de Patton.[107]

Chez l'adulte, la surface de contact des conduits aériens ne mesure que quelques mètres carrés, alors que celle des alvéoles peut dépasser les 100 $m^2$.[131, 132] Outre le grand écart en termes de surface de contact entre les deux principales zones fonctionnelles des poumons, la composition cellulaire de leur épithélium est nettement différente. Parmi les cellules qui composent l'épithélium au niveau de la zone de conduction, on y trouve principalement les cellules basales, les cellules caliciformes, les cellules ciliées et les cellules en brosse (Figure **14**). Les cellules basales, cellules souches de l'épithélium, sont capables de se différencier en cas de blessure ou d'apoptose. Les cellules en brosse, quant à elles, sont impliquées dans le métabolisme des médicaments. Les cellules caliciformes sécrètent un mucigène qui se transforme en mucus après hydratation, alors que les cellules ciliées fournissent le mécanisme de déplacement à la couche de mucus formée. La combinaison des cellules caliciformes et ciliées forme le tapis muco-ciliaire qui représente un mécanisme de défense important pour l'organisme. À chaque

inspiration, le mouvement ciliaire agit en empêchant les particules insolubles inhalées (ex. poussières) de progresser en leur permettant de remonter efficacement vers la trachée.[133]

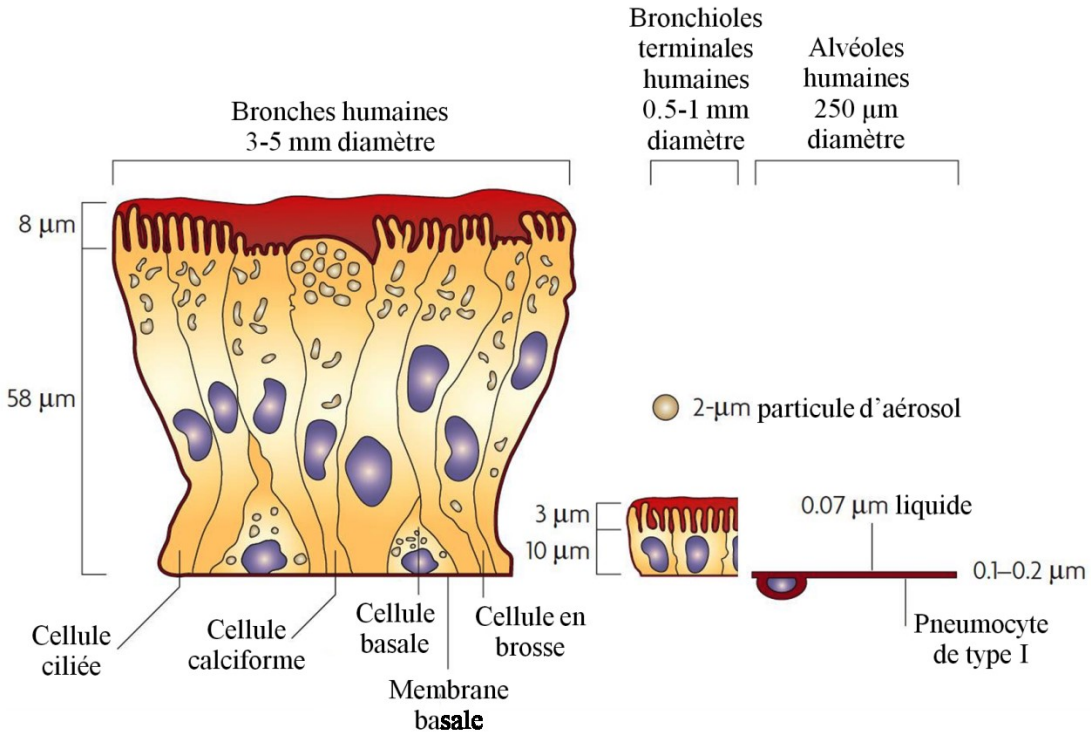


**Figure 14**. Composition cellulaire de l'épithélium pulmonaire aux différents sites dans les poumons. Adaptée de Patton.[107]

Ainsi, cette composition cellulaire de l'épithélium pulmonaire persiste jusqu'aux petites voies aériennes, en s'amincissant progressivement jusque dans l'arbre pulmonaire profond. Une fois dans les alvéoles, la composition cellulaire de l'épithélium est complètement différente. À ce niveau, plus de 95-97% de la surface alvéolaire devient occupée par des pneumocytes de type I, très larges et extrêmement minces (<0.1 µm à certains endroits), formant la paroi alvéolaire. Leur rôle principal est d'offrir une barrière d'épaisseur minimale facilement perméable aux gaz, tels que l'oxygène et le dioxyde de carbone. Le 3-5% restant de la surface alvéolaire est constitué de pneumocytes de type II responsables de sécréter le surfactant qui permet de faciliter les échanges gazeux et diminuer la tension de surface de la paroi. Les pneumocytes de type

II peuvent aussi agir comme cellules progénitrices capables de se différencier en pneumocytes de type I ou II, au besoin.[134]

## 5.3.2. Propriétés physico-chimiques des médicaments inhalés

La membrane plasmique de l'épithélium pulmonaire représente la barrière de résistance principale à l'absorption des médicaments administrés par voie pulmonaire.[135, 136] Les propriétés physico-chimiques des particules d'aérosol générées par les dispositifs d'inhalation jouent un rôle déterminant dans le processus d'absorption des médicaments inhalés. Une fois délivrées et déposées dans les poumons, les particules d'aérosol se mettent en solution sur les surfaces des voies aériennes et la vitesse d'absorption ($v_{abs}$) peut être décrite par la relation suivante:[137]

$$v_{abs} = P \times A \times C$$

*P*: La perméabilité membranaire au médicament (directement proportionnelle à son coefficient de partage et inversement proportionnelle à l'épaisseur de la membrane).
*A*: Aire ou surface de contact disponible pour l'absorption du médicament.
*C*: Concentration de médicament (répartie sur la surface d'absorption).

### 5.3.2.1. Coefficient de partage octanol/eau (Log P)

La demi-vie d'absorption pulmonaire d'un médicament (temps requis pour que 50% du médicament soit absorbé) peut être estimée à partir du Log P.[138, 139] Les médicaments de Log P >1 sont absorbés avec une demi-vie d'absorption de l'ordre d'une minute. Au fur et à mesure que cette valeur diminue et atteint un Log P ≤-1, la demi-vie d'absorption augmente graduellement pour ces médicaments.[140, 141] Les petites molécules, légèrement hydrophobes, peuvent donc présenter une cinétique d'absorption extrêmement rapide dans les poumons.[142]

### 5.3.2.2. Poids moléculaire (PM)

En général, plus le poids moléculaire d'une molécule est faible, plus elle sera absorbée rapidement par les poumons. Ainsi, alors que les médicaments à faible poids moléculaire peuvent être absorbés en quelques secondes à quelques minutes, ceux avec un poids moléculaire plus élevé (protéines solubles) peuvent prendre des heures, jours ou

semaines avant d'être absorbés. Par contre, contrairement au tractus gastro-intestinal pratiquement imperméable à la plupart des molécules >600 Da, l'épithélium pulmonaire est doté de mécanismes de transport pouvant assurer l'absorption de molécules atteignant jusqu'à 160 000 Da. De manière générale, les petites molécules hydrophobes sont celle les plus rapidement absorbées à travers la membrane plasmatique de l'épithélium pulmonaire (diffusion passive), tandis que les petites molécules hydrophiles le sont un peu moins rapidement via la voie paracellulaire (jonctions serrées prédominantes dans la zone de conduction) ou les transporteurs spécifiques.[132, 142]

### 5.2.3. Propriétés physico-chimiques de la milrinone

La milrinone est une molécule de relativement petite taille ($C_{12}H_9N_3O$ PM: 211.2) dont la structure chimique (Figure **15**) est dérivée de la famille des bipyridines (1,6-Dihydro-2-methyl-6-oxo-[3,4′-bipyridine]-5-carbonitrile).

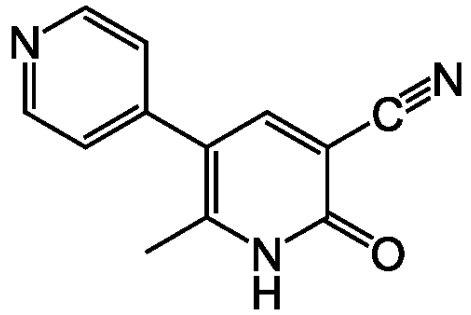


**Figure 15**. Structure chimique de la milrinone. Tirée de Wikimedia.

Son Log P est de 1.17 et son pKa peut être de 4.5 et 8.5. La milrinone est soluble dans les solutions aqueuses acide ou basique, le méthanol et le chloroforme. La milrinone est disponible commercialement sous forme de lactate de milrinone ($C_{12}H_9N_3O$, $C_3H_6O_3$ PM: 301.3) en ampoule de 10 ml contenant l'équivalent de 1 mg de lactate de milrinone et 47 mg de dextrose anhydre USP par ml d'eau pour injection (Milrinone Lactate Injection, Primacor®). La solution injectable a un pH ajusté entre 3.2 et 4.0 avec de l'acide lactique ou de l'hydroxyde de sodium, la concentration lactate de milrinone totale pouvant varier entre 0.95 et 1.29 mg/ml.

## 5.4. Propriétés pharmacocinétiques des médicaments inhalés

Les poumons sont robustes et capables de supporter l'exposition chronique à un grand nombre de médicaments inhalés approuvés par Santé Canada. Contrairement à la voie orale, l'administration par voie pulmonaire permet aux petites molécules d'être absorbées beaucoup plus rapidement, voir presqu'instantanément, en l'absence d'un métabolisme important des médicaments.

### 5.4.1. Absorption pulmonaire

Les poumons représentent la voie d'administration la plus rapide après la voie intraveineuse.[142] Étant donné la grande surface des poumons, la grande perméabilité de l'épithélium pulmonaire et la capacité de dispersion d'un aérosol,[132] les petites molécules déposées dans les poumons à chaque inspiration sont presqu'instantanément absorbées dans la circulation systémique. La prochlorpérazine[143] (Figure **16**), la morphine[144] (Figure **17**) et le fentanyl sont des exemples de médicaments inhalés qui présentent des cinétiques d'absorption très rapide, de l'ordre de quelques secondes. Leur profil pharmacocinétique suite à une administration par voie inhalée est pratiquement superposable à celui suivant une administration par voie intraveineuse.

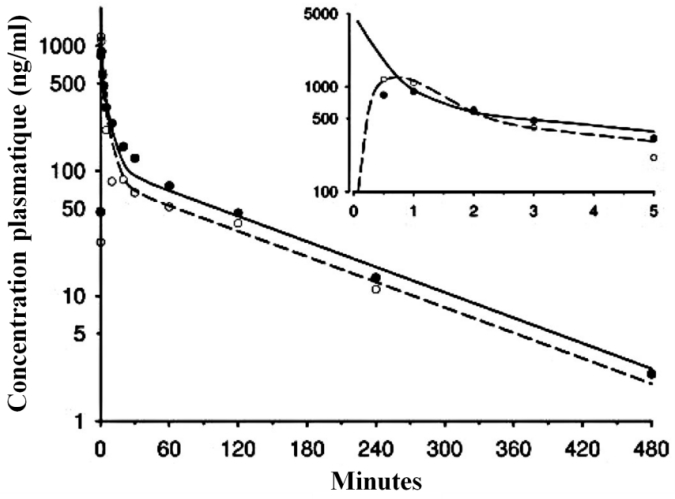


**Figure 16**. Profils pharmacocinétiques de la prochlorpérazine suite à une administration par voie inhalée (symbole vide) et par voie intraveineuse (symbole plein) chez le chien. Adaptée de Avram.[143]

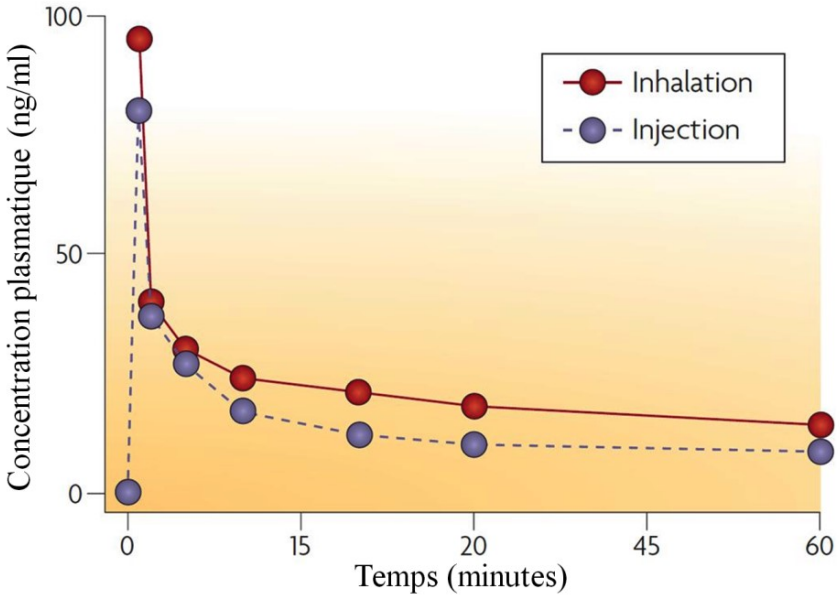


**Figure 17**. Profils pharmacocinétiques de la morphine suite à une administration par voie inhalée (cercle rouge) et par voie intraveineuse (cercle mauve) chez les volontaires sains. Adaptée de Patton.[107]

## 5.4.2. Métabolisme pulmonaire

Chez l'adulte, la surface interne de chacune des 500 millions alvéoles pulmonaires renferme environ 12-14 macrophages qui assurent continuellement la séquestration et la digestion rapide des particules insolubles déposées dans les alvéoles.[145] Les particules d'aérosol ayant la capacité d'être rapidement absorbées à travers l'épithélium pulmonaire peuvent éviter, en grande partie, cette dégradation par les macrophages. Par ailleurs, la plupart des enzymes responsables du métabolisme des médicaments sont présents à des concentrations beaucoup plus faibles dans les poumons comparativement au tractus gastro-intestinal et au foie.[146-148] Par conséquent, les molécules inhalées sont moins susceptibles à la dégradation lors de leur cheminement vers la circulation systémique, contrairement à la voie orale ou une autre voie extravasculaire. La nicotine, le Δ-9-tetrahydrocannabinol (THC), le rizatriptan et la testostérone sont des exemples de médicaments qui réussissent à obtenir une forte biodisponibilité systémique en étant rapidement absorbés après inhalation. Ainsi, les poumons représentent la voie d'administration non invasive offrant la meilleure biodisponibilité systémique pour les

grosses[132, 144] et les petites[132, 149, 150] molécules en leur offrant la possibilité d'atteindre une biodisponibilité systémique de près de 100%. L'inhalation représente donc un moyen fiable et efficace pour administrer les médicaments sous forme d'aérosols leur permettant d'éviter le métabolisme de premier passage intestinal et hépatique ou encore un effet postprandial risquant de diminuer leur biodisponibilité systémique.

# CHAPITRE 6: Pharmacodynamie des médicaments

La pharmacodynamie (PD) étudie l'effet du médicament suite à son administration dans l'organisme, en d'autres mots « ce que le médicament fait à l'organisme ». Elle s'intéresse aux mécanismes d'action du médicament dans l'organisme, ainsi qu'à l'évolution temporelle de l'effet du médicament, tel que le début, l'intensité et la durée de l'effet. La PD cherche à décrire la relation entre les concentrations du médicament et l'effet (désirable ou indésirable) observé.

Le mécanisme d'action du médicament est généralement bien connu et repose sur le principe d'une liaison ligand-récepteur, où le ligand est le médicament et le récepteur est le site d'action du médicament e.g. un récepteur cellulaire, une enzyme, un canal ionique, etc. Selon l'affinité du médicament pour son récepteur, la liaison déclenchera une transduction et une cascade de signalisation intracellulaire associées à une intensité de l'effet observé.

$$[Médicament] + [Récepteur] \leftrightarrow [Médicament - Récepteur] \rightarrow Effet$$

L'interaction entre le médicament et son récepteur peut avoir un effet activateur (médicament agoniste) ou inhibiteur (médicament antagoniste) et celle-ci sera fonction de la concentration du médicament au site d'action ainsi que de son affinité pour le récepteur. Il est important de faire la distinction entre l'action pharmacologique, l'effet pharmacologique et la réponse thérapeutique. L'action pharmacologique est la liaison du médicament à son récepteur (site d'action) et le délai d'action reflète le temps requis au médicament pour d'atteindre son site d'action (accessibilité du récepteur, débit sanguin, processus ADME). L'effet pharmacologique est une conséquence observable de l'action pharmacologique et peut être retardé s'il dépend de médiateurs physiologiques intermédiaires. La réponse thérapeutique résulte de l'effet pharmacologique chez un individu malade ou non et correspond au phénomène clinique observé et à l'objectif thérapeutique visé.

Ainsi, pour une dose de médicament donnée, l'effet peut être quantifié en fonction des concentrations mesurées selon la relation suivante:

$$E = \frac{E_{max} \cdot C}{EC_{50} + C}$$

où *E* représente l'effet pharmacologique du médicament et $EC_{50}$, la concentration de médicament produisant 50% de l'effet maximal ($E_{max}$) dans la biophase. Lorsque l'effet pharmacologique est tracé en fonction des concentrations plasmatiques sur un graphique semi-logarithmique, une courbe concentration-effet en forme de sigmoïde peut être observée (Figure **18**).

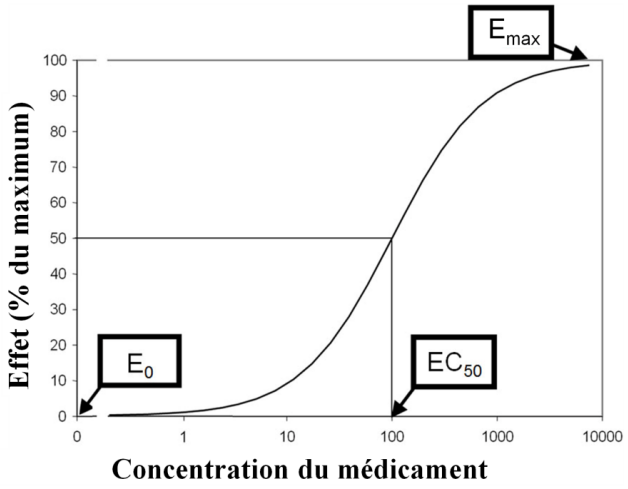


**Figure 18**. Relation concentration-effet des médicaments. $E_0$, effet au temps zéro (valeur de référence); $EC_{50}$, concentration de médicament produisant 50% de l'effet maximal ($E_{max}$). Adaptée de Holford.[151]

L'effet mesuré peut être exprimé sous forme de valeur absolue ou de valeur relative en pourcentage de $E_{max}$. L'effet observé au temps zéro ($t_0$) est reconnu comme étant l'effet observé à l'état basal « baseline » ($E_0$) et correspond à la valeur de référence en l'absence du médicament. Les deux principales propriétés pharmacodynamiques d'un médicament sont le $E_{max}$ et le $EC_{50}$, qui reflètent respectivement l'efficacité et la puissance du médicament. Ainsi, un médicament sera d'autant plus efficace que son $E_{max}$ sera important ($EC_{50}$ inchangé) et d'autant plus puissant que son $EC_{50}$ sera faible ($E_{max}$ inchangé).

## 6.1. Démonstration de l'efficacité d'un médicament

Dans le contexte du développement du médicament, les essais cliniques de phase II ont pour objectif de démontrer l'efficacité du nouveau traitement chez les patients. Les études cliniques de phase II, généralement subdivisées en deux phases (IIa et IIb), consistent notamment à évaluer l'innocuité et la PK, tester la pharmacologie et l'efficacité clinique, établir les relations exposition-réponse et PK/PD et enfin déterminer la dose optimale (posologie) pour les études de phase III. On distingue trois types de paramètres d'efficacité utilisés pour évaluer l'effet du médicament: les biomarqueurs, les issues cliniques et les paramètres substituts.[152]

- **Biomarqueur « biomarker »:** Mesure objective (mesure de laboratoire, signe physique) d'un processus biologique normal, d'un processus pathologique, ou d'une réponse pharmacologique au traitement.
- **Issue clinique « clinical endpoint »:** Mesure directe d'un résultat clinique (comment le patient se sent, fonctionne ou survit).
- **Paramètre substitut « surrogate marker »**: Biomarqueur validé par des études épidémiologiques pouvant se substituer à une issue clinique et dont la réponse au traitement corrèle (correspond) avec celle de l'issue clinique.

En règle générale, les biomarqueurs ne répondent pas directement à l'objectif thérapeutique (efficacité clinique), mais documentent les mécanismes d'action des médicaments (preuve de concept). Durant la phase IIa, ils sont utilisés pour leur caractère plus informatif et servent à établir les relations exposition-réponse. Durant la phase IIb, les issues cliniques sont également utilisées pour démontrer le bénéfice clinique (pas toujours possible). Lors des études cliniques, l'utilisation des biomarqueurs présente certains avantages par rapport à ceux des issues cliniques: ils représentent des mesures objectives faciles à interpréter et impliquent des études moins coûteuses, de plus courte durée et nécessitant moins de patients (Tableau **V**).

**Tableau V**. Démonstration de l'efficacité d'un médicament au cours des études cliniques de phases II et III.

| | **Phase IIa** | **Phase IIb-III** |
|---|---|---|
| **Taille d'échantillon** | Petite | Grande |
| **Paramètre d'efficacité** | Biomarqueur | Issue clinique ou paramètre substitut |
| **Démonstration** | Preuve de concept | Efficacité clinique |

Lors de la planification d'un essai clinique, le modèle mécanistique décrivant les étapes successives nécessaires à l'obtention de la réponse thérapeutique suite à l'administration d'un médicament se compose des modèles suivants (Figure **19**): un modèle PK (dose–exposition), un modèle PK/PD (exposition–réponse) et un modèle réponse–issue clinique.

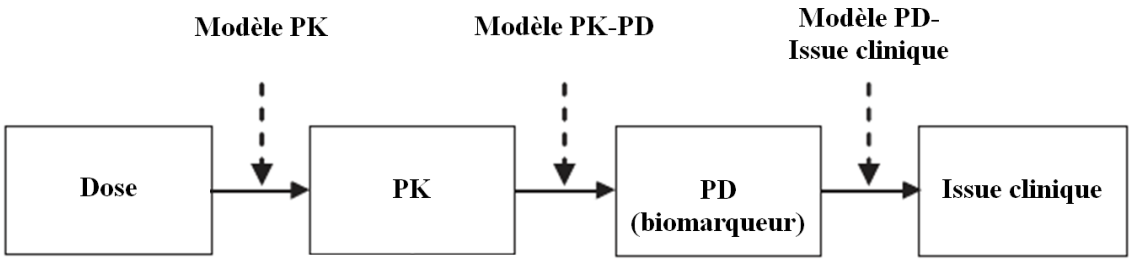


**Figure 19**. Modèle mécanistique de la relation causale entre la pharmacocinétique (PK) et la pharmacodynamie (PD).

Par définition, un paramètre substitut est un biomarqueur validé pour être utilisé à la place d'une issue clinique (mesure directe de l'efficacité clinique). Toutefois, un biomarqueur n'est pas forcément validé comme un paramètre substitut même s'il est fortement lié au mécanisme physiopathologique de la maladie et au mécanisme d'action du médicament. Selon les agences réglementaires,[153] deux conditions essentielles sont utilisées pour valider un biomarqueur comme paramètre substitut:

1. le biomarqueur doit être corrélé avec l'issue clinique
2. le biomarqueur doit capter entièrement l'effet net du traitement sur l'issue clinique (très difficile à vérifier)

La validation du paramètre substitut permet de le qualifier comme un test diagnostique de l'effet du traitement sur l'issue clinique, *i.e.*, un changement induit par le traitement sur le paramètre substitut devrait être reflété par un changement correspondant sur l'issue clinique. Non seulement la valeur prédictive du paramètre substitut doit être validée (sensibilité, spécificité, etc.), elle doit également être facilement disponible et facilement mesurable. Dans notre cas, le ratio PAm/PAPm représenterait un candidat potentiel en tant que paramètre substitut, puisqu'il découle de mesures de pression prises d'emblée en chirurgie cardiaque et aisément obtenues avant l'initiation de la CEC (valeur pronostique).

La validation du biomarqueur en tant que paramètre substitut doit être confirmée par plusieurs études:[154]

- Études exploratoires supportant la plausibilité biologique de la relation entre le biomarqueur et l'issue clinique.
- Études épidémiologiques expérimentales vérifiant la capacité de prédiction du biomarqueur pour l'issue clinique.
- Études cliniques démontrant que le médicament exerce des effets correspondants sur le biomarqueur et l'issue clinique.

## 6.2. Démonstration de l'efficacité de la milrinone

Dans le cas de la milrinone inhalée, le traitement à l'étude constitue à la fois une nouvelle indication (hypertension pulmonaire) et une nouvelle voie d'administration (inhalation) pour un médicament déjà existant. L'objectif de notre projet de recherche consiste à démontrer l'efficacité de la milrinone inhalée dans la prévention d'une sortie de CEC difficile (issue clinique). Parmi plusieurs paramètres hémodynamiques utilisés en chirurgie cardiaque, le ratio PAm/PAPm a été sélectionné pour servir de biomarqueur PD.

Dans une étude portant sur 1439 patients consécutifs en chirurgie cardiaque, le ratio PAm/PAPm s'est avéré comme le paramètre hémodynamique le plus significatif dans la prédiction d'un index composite de décès, d'arrêt cardiaque post-op, de besoin de support mécanique post-op et de besoin de support vasoactif excédant 24 heures post-

op.[31] De plus ce ratio est le meilleur prédicteur de l'indice d'excentricité (IE) du septum interventriculaire.[155] Cet indice est le reflet de l'effet des pressions intraventriculaires droites et gauches sur le septum interventriculaire. L'IE est donc associé à la sévérité de l'hypertension pulmonaire et de ses effets sur le ventricule droit. D'autres études[71, 156] et un rapport de cas[78] en chirurgie cardiaque ont également noté qu'une augmentation du ratio PAm/PAPm observée suite à l'administration d'agents inhalés tels que la milrinone était associée à une amélioration de la fonction ventriculaire droite. Pour ces raisons, le ratio PAm/PAPm a été sélectionné pour servir de biomarqueur PD et de paramètre intermédiaire dans l'établissement de la relation causale entre l'exposition et la réponse à la milrinone inhalée (Figure **20**).

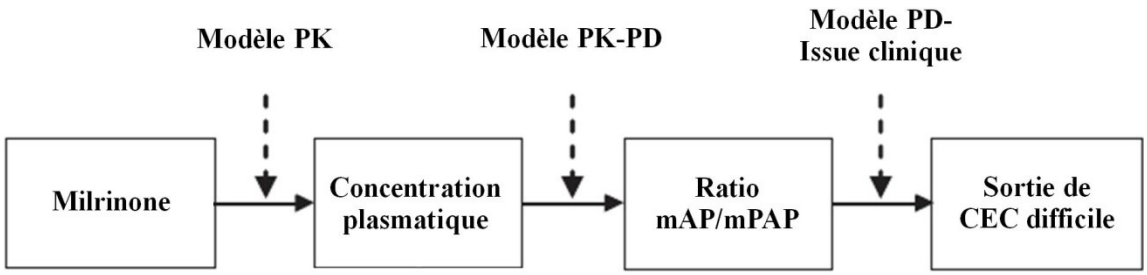


**Figure 20**. Modèle mécanistique de la relation causale appliquée à la milrinone. PK, pharmacocinétique; PD, pharmacodynamie; PAm, pression artérielle moyenne; PAPm, pression artérielle pulmonaire moyenne; CEC, circulation extracorporelle.

# CHAPITRE 7. Modélisation pharmacocinétique/pharmacodynamique

L'évolution temporelle de l'action du médicament combine les principes de la pharmacocinétique et de la pharmacodynamie. La modélisation PK/PD représente un outil intéressant permettant de faire le lien entre la partie PK et la partie PD pour caractériser les variations de l'effet en fonction des concentrations sanguines mesurées et prédire l'évolution des effets dans le temps (Figure **21**).

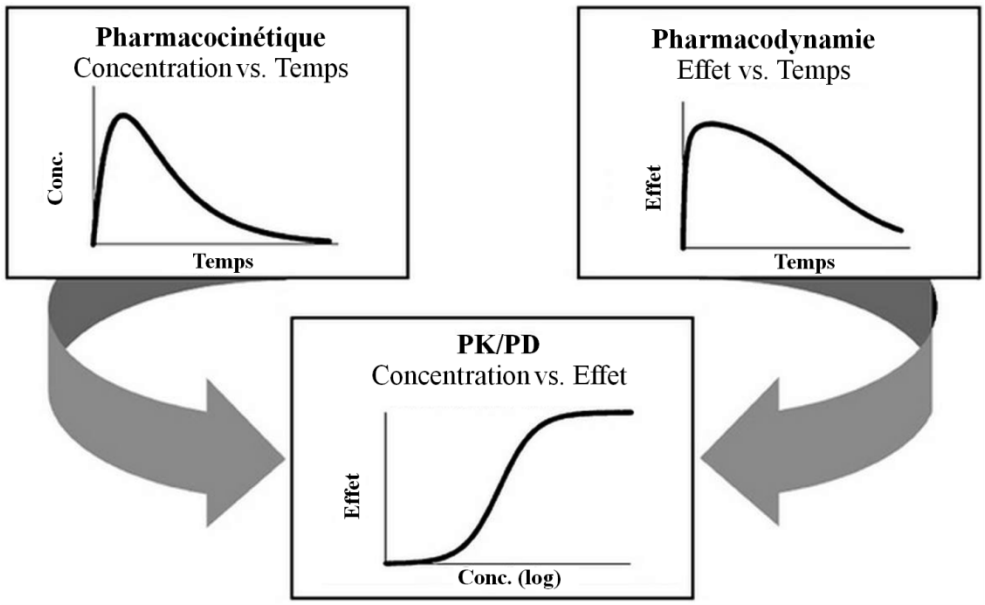


**Figure 21**. Concept général de la modélisation pharmacocinétique/pharmacodynamique (PK/PD). Adaptée de Mehrotra.[157]

De manière générale, la modélisation PK/PD intégre la PK et la PD dans une série d'expressions mathématiques permettant de décrire et prédire l'évolution temporelle de l'effet (intensité) en réponse à l'administration d'un médicament (dose). Le modèle PK permet de prédire les concentrations plasmatiques au même temps que les données recueillies pour la pharmacodynamie. Le modèle PK/PD final permet de prédire les concentrations au site d'action de même que l'effet mesuré en fonction de ces concentrations. L'objectif principal de la modélisation PK/PD est de caractériser l'efficacité du médicament afin de déterminer la concentration minimale efficace dans

une catégorie de patients donnée. Cette information est importante pour établir des régimes posologiques préservant l'efficacité et l'innocuité du médicament. De plus, les modèles PK et/ou PD peuvent servir à simuler de nouvelles données à partir de changements des conditions reliées à l'administration du médicament (dose, posologie) ou à l'état du patient (physiologique ou pathologique).

Enfin, la modélisation consiste à estimer des paramètres PK et/ou PD servant à décrire de manière vraisemblable les phénomènes observés en se voulant de respecter les principes suivants:[158]

- ✓ Adaptation adéquate aux besoins de l'utilisateur
- ✓ Développement basé sur des fondements physiologiques et scientifiques
- ✓ Description des phénomènes observés avec une précision et exactitude
- ✓ Principe de parcimonie: Modèle simple, i.e. nombre minimum de paramètres

L'analyse individuelle exige une collecte rigoureuse de données riches qui sont nécessaires à la détermination des paramètres pharmacocinétiques tels: $Cl$, $V_d$, $ASC$, $C_{max}$, $t_{max}$ et $t_{1/2}$. Selon les présuppositions de base requises pour l'analyse des données, l'estimation des paramètres pharmacocinétiques peuvent dériver d'une analyse compartimentale ou non compartimentale.

## 7.1. Analyse compartimentale

L'analyse compartimentale est une approche quantitative qui consiste à réduire l'organisme en un ou plusieurs compartiment(s) pour établir un modèle structural permettant d'expliquer adéquatement les phénomènes observés. Un compartiment peut être défini comme un espace virtuel dans lequel un médicament (principe actif) est distribué de manière homogène.[80] La plupart du temps, le modèle structural compartimental est composé d'un, deux ou trois compartiments pour décrire les données observées. Le premier compartiment, *compartiment central*, représente normalement l'ensemble des tissus hautement perfusés (foie, reins et poumons) considérés en équilibre avec la circulation systémique. Les compartiments périphériques représentent les autres tissus plus faiblement perfusés (tissus adipeux, muscles et peau).

### 7.1.1. Paramètres pharmacocinétiques

Chaque compartiment possède un volume apparent ($V_x$) dans lequel le médicament peut se distribuer et des constantes de vitesse ($k_{xx}$) gérant les entrées et les sorties du médicament au niveau du compartiment.[159] Les constantes de vitesse peuvent représenter un processus d'absorption, d'élimination ou de transfert d'ordre 1 (vitesse varie avec les concentrations; diffusion passive), d'ordre 0 (vitesse indépendante des concentrations; transport actif saturé dont la vitesse est devenue constante) ou non linéaire.

Ces constantes sont régies par un système d'équations différentielles dont la solution intégrée permet de décrire mathématiquement l'évolution des concentrations plasmatiques du médicament en fonction du temps (Figure **22**).

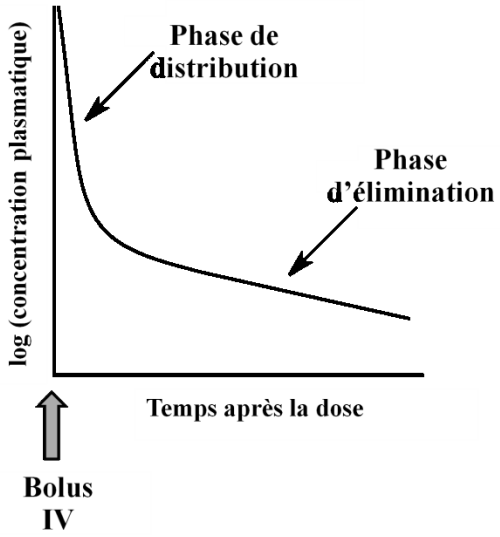


**Figure 22**. Profil pharmacocinétique d'un modèle structural à deux compartiments. IV, intraveineux. Adaptée de Wikimedia.

Par exemple, dans le cas d'un modèle structural à deux compartiments suite à une administration intraveineuse (Figure **23**), le volume de distribution central ($V_1$), les constantes de vitesse de transfert ($k_{12}$, $k_{21}$) et la constante de vitesse d'élimination ($k_{10}$) sont des micro-constantes servant à écrire le système d'équations différentielles.

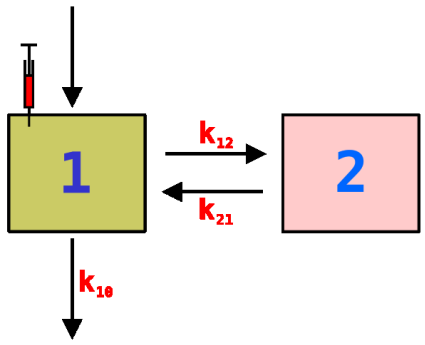


**Figure 23**. Modèle structural à deux compartiments suite à une administration intraveineuse. $k_{12}$ et $k_{21}$, constantes de vitesse de transfert; $k_{10}$, constante de vitesse d'élimination. Tirée de Wikimedia.

La solution intégrée de ce système d'équations différentielles permet d'exprimer les macro-constantes ($A$, $B$, $\alpha$ et $\beta$) pour décrire l'évolution temporelle des concentrations plasmatiques du médicament:[80]

$$\hat{C}_p = A \cdot \mathrm{e}^{-\alpha t} + B \cdot \mathrm{e}^{-\beta t}$$

où la variable dépendante $\hat{C}_p$ représente les concentrations prédites dans le compartiment central au temps de la variable indépendante $t$, les coefficients $A$ et $B$ correspondent aux concentrations prédites dans le compartiment central au temps zéro pour les phases de distribution ($\mathrm{e}^{-\alpha t}$) et d'élimination ($\mathrm{e}^{-\beta t}$), respectivement. Les constantes α et β sont les paramètres estimés de vitesse de décroissance pour chacune de ces phases et permettent de calculer les temps de demi-vie de distribution ($t_{1/2\,\alpha}$) et d'élimination ($t_{1/2\,\beta}$).

Les paramètres utilisés pour caractériser le modèle peuvent donc être exprimés en termes de micro-constantes ($V_1$, $k_{10}$, $k_{12}$, $k_{21}$), de macro-constantes ($A$, $B$, $\alpha$ et $\beta$) ou de volumes et clairances ($V_1$, $V_2$, $Cl$ et $Q$). Les uns peuvent être estimés à partir des autres et vice versa.

### 7.1.2. Estimation des paramètres

La modélisation repose donc sur l'estimation adéquate des paramètres PK et/ou PD permettant de décrire et prédire correctement les données observées. Pour ce faire, les

logiciels de modélisation utilisent des algorithmes d'estimation appliqués aux modèles non linéaires et fournissent des paramètres estimés.

Dans l'exemple précédent du modèle pharmacocinétique à deux compartiments suite à une administration intraveineuse ($\hat{C}_p = A \cdot \mathrm{e}^{-\alpha t} + B \cdot \mathrm{e}^{-\beta t}$), il s'agit d'un modèle de régression non linéaire en ce qui concerne les paramètres $A$, $B$, $\alpha$ et $\beta$ (macro constantes) vis-à-vis la variable dépendante $\hat{C}_p$. Les quatre paramètres sont donc estimés en appliquant des modèles de régression non linéaires de manière à minimiser les résidus, ie. la différence entre les concentrations observées ($C_p$) et celles prédites ($\hat{C}_p$) par le modèle (Figure **24**).

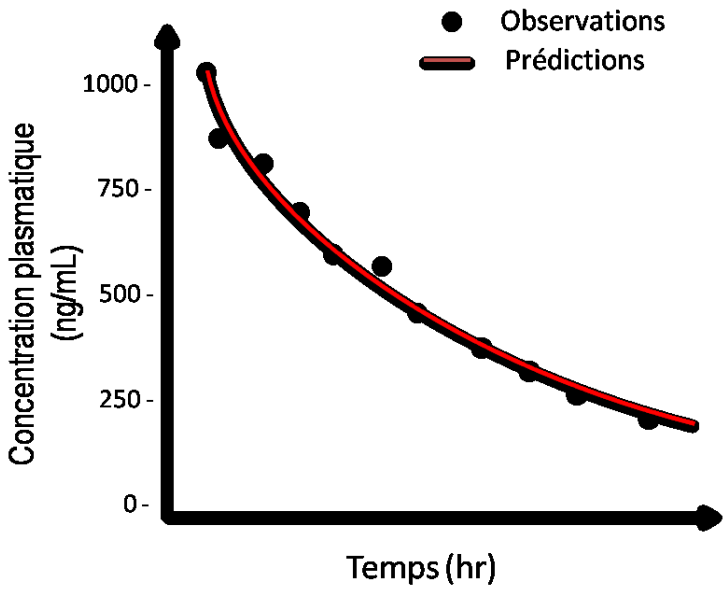


**Figure 24**. Concentrations plasmatiques observées et prédites en fonction du temps. Avec permission de Gaudreault.

La fonction objective « objective function » (*OF*) est un critère important permettant d'évaluer la qualité du lissage (ajustement) des données observées par un modèle. La *OF* est définie comme étant la somme des moindres carrés de la différence entre les valeurs observées (*obs*) et prédites (*pred*):

$$OF = \sum_{i=1}^{n} W_i (obs_i - pred_i)^2$$

où $i$ est un nombre de 1 à $n$, $n$ le nombre de prélèvements (e.g. couples temps et concentration), et $W_i$ le poids appliqué à la différence au carré.

Les méthodes de minimisation de la *OF* les plus communément utilisées sont: le critère des moindres carrés ordinaires (« ordinary least squares », *OLS*), le critère des moindres carrés pondérés (« *weighted least squares », WLS*) ou des moindres carrés généralisés (« *generalized least squares », GLS*) et le critère des moindres carrés étendus (« *extended least squares », ELS*) ou du maximum de vraisemblance (*« Maximum Likehood »*). Ces méthodes diffèrent entre elles au point de vue du choix de poids $W_i$ appliqué à la fonction.[158]

Dans le processus de sélection du modèle final, plusieurs critères d'évaluation visuelle[160] et statistique[161] permettent à l'utilisateur de faire un choix éclairé, à savoir si le modèle converge vers la bonne solution.

- ✓ Graphiques diagnostiques: Évaluation de la qualité du lissage des données par le modèle structural (1, 2 ou 3 compartiments) et choix du poids $W_i$ à appliquer au modèle (analyse des résidus)
- ✓ Valeur et coefficient de variation: Évaluation de l'exactitude et de la précision des paramètres
- ✓ Matrice de corrélation: Évaluation de la corrélation entre les paramètres
- ✓ Nombre de conditionnement: Évaluation de la stabilité du modèle
- ✓ Test de F: Comparaison de deux modèles rivaux hiérarchiques
- ✓ Critère d'Akaike: Comparaison de deux modèles rivaux non hiérarchiques

## 7.2. Analyse non compartimentale

L'analyse non compartimentale est dite modèle-indépendante puisque, contrairement à l'analyse compartimentale, elle ne nécessite pas de présupposition au niveau du nombre de compartiments.[80]. En fait, l'organisme est considéré comme un seul espace (compartiment) dans lequel le médicament est administré, d'où l'échantillonnage est effectué et duquel l'élimination (ordre 1) seulement se produit. Cette méthode d'analyse

utilise des équations algébriques simples qui permettent une approche descriptive des phénomènes observés.

### 7.2.1. Pharmacocinétique

Lorsque les concentrations plasmatiques ($C_p$) sont représentées en fonction du temps ($t$) sur un graphique semi-logarithmique, la pente terminale correspond à la constante de vitesse d'élimination ($k_e$) dans un modèle monocompartimental, souvent dénotée $\lambda_z$ en analyse non compartimentale (Figure **25**). Afin de bien caractériser la demi-vie d'élimination d'un médicament, il est important qu'un nombre suffisant d'échantillons soient prélevés (minimum 3-4 prélèvements) couvrant une période de temps assez longue (minimum 2-4 demi-vies).

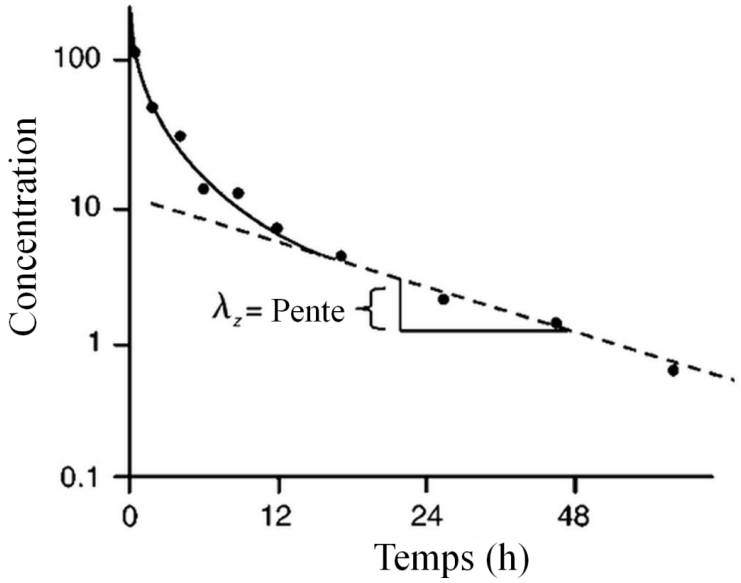


**Figure 25**. Estimation de la constante de vitesse d'élimination $\lambda_z$. Adaptée de Takimoto.[162]

L'aire sous la courbe ($ASC$) des concentrations plasmatiques ($C_p$) en fonction du temps ($t$) représente l'exposition globale (ou cumulative) de médicament dans l'organisme (Figure **26**).

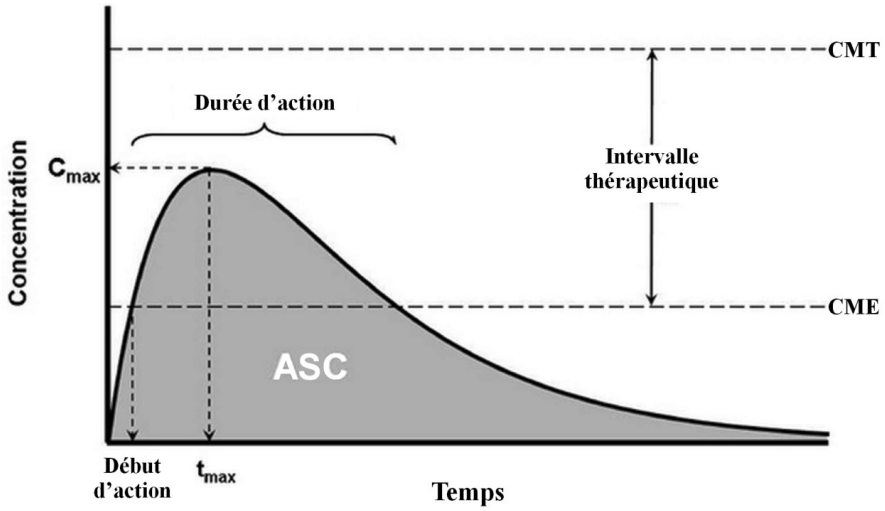


**Figure 26**. Les paramètres pharmacocinétiques décrivant un profil typique des concentrations plasmatiques en fonction du temps suivant une administration orale. $C_{max}$, concentration maximale; $t_{max}$, temps de $C_{max}$; ASC, aire sous la courbe; CMT, concentration maximale tolérée; CME, concentration minimale efficace. Adaptée de Mehrotra.[157]

Dans un système linéaire, i.e. où la *Cl* du patient est constante, l'exposition de l'organisme au médicament est proportionnelle à la dose administrée (si *F* est constante):

$$ASC_{0\rightarrow\infty} = \frac{F \cdot D}{Cl}$$

où $ASC_{0\rightarrow\infty}$ représente l'aire sous la courbe depuis l'administation du médicament ($t_0$) jusqu'à l'infini ($t_\infty$).

La méthode des trapèzes (Figure **27**) permet d'estimer l'*ASC* tout au long de la période d'échantillonnage, soit de $t_0$ jusqu'au temps du dernier prélèvement $t_{last}$ ($ASC_{0\rightarrow tlast}$) et consiste à faire la sommation des aires d'un très grand nombre de trapèzes dont la base correspond à l'intervalle de temps entre deux prélèvements ($\Delta t$). Plus les intervalles sont petits, plus l'estimation sera précise.

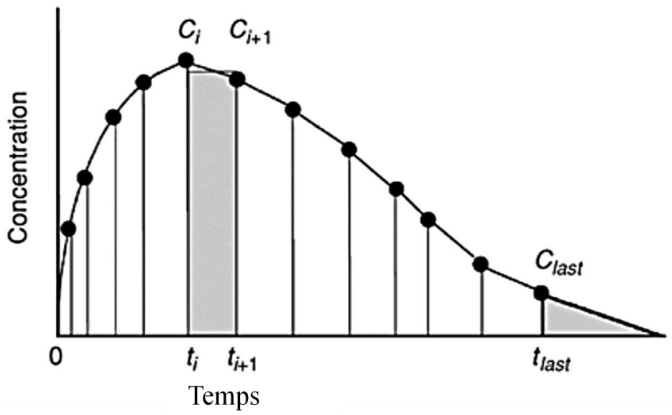


**Figure 27**. Estimation de l'aire sous la courbe des concentrations (*C*) en fonction du temps (*t*) en utilisant la règle trapézoïdale linéaire-linéaire. $C_{last}$, concentration du dernier prélèvement; $t_{last}$, temps du dernier prélèvement. Adaptée de Takimoto.[162]

L'expression mathématique de l'$ASC_{0 \rightarrow t}$ par cette méthode peut être simplifiée ainsi:

$$ASC_{0 \rightarrow \mathrm{t}} = \sum_{i=1}^{n} \left[ \frac{C_i + C_{(i+1)}}{2} \cdot (t_{(i+1)} - t_i) \right]$$

où *i* est un nombre de 1 à *n*, et *n* le nombre de prélèvements (couples temps et concentration).

Pour ce qui est de la partie extrapolée de l'ASC depuis le dernier prélèvement jusqu'à l'infini ($ASC_{tlast \rightarrow \infty}$), elle peut être calculée comme suit:

$$ASC_{\mathrm{tlast} \rightarrow \infty} = \frac{C_{last}}{k_e}$$

où $k_e$ est la constante de vitesse d'élimination, et $C_{last}$ la concentration du dernier prélèvement.

De cette manière, on obtient:

$$ASC_{0 \rightarrow \infty} = \sum_{i=1}^{n} \left[ \frac{C_i + C_{(i+1)}}{2} \cdot (t_{(i+1)} - t_i) \right] + \frac{C_{last}}{k_e}$$

### 7.2.2. Pharmacodynamie

En pharmacodynamie, une réponse clinique observée (désirable ou indésirable) peut être le résultat de l'action cumulée du médicament dans l'organisme. Dans cette situation, l'effet cumulatif du médicament est obtenu en calculant l'aire sous la courbe de l'effet en fonction du temps (ASCE) par la méthode des trapèzes. Une estimation plus précise de l'effet global du médicament est ainsi obtenue en intégrant l'effet en fonction du temps plutôt qu'en regardant les mesures individuelles de l'effet.[163]

Dans le cas d'un médicament exogène (qui n'est pas produit par l'organisme), la valeur de base de l'ASC calculée durant l'analyse PK est égale à zéro, i.e., la concentration initiale est égale à zéro et la concentration finale est de retour à zéro une fois le médicament totalement éliminé de l'organisme. Toutefois, lorsque la valeur de base est différente de zéro (e.g., dans le cas d'une substance endogène lors du calcul de l'ASC ou dans le cas d'une réponse pharmacologique lors du calcul de l'ASCE), ce qui nous intéresse est l'amplitude du changement par rapport à la ligne de base. En effet, puisque la réponse d'intérêt a une valeur de référence non nulle, celle-ci doit être prise en compte dans l'estimation de la réponse pharmacologique net (ASCE net) en quantifiant la réponse d'intérêt qui dévie (augmentation et diminution) de la valeur de base. Cet effet cumulatif peut être utilisé pour prédire la réponse thérapeutique et sera affecté par des changements au niveau de l'exposition au médicament. Cette approche est entre autres utile pour évaluer l'effet net pour une dose de médicament donnée.[164]

# CHAPITRE 8. Objectifs et hypothèse de recherche

## 8.1. Objectifs généraux et hypothèse de recherche

L'hypothèse de recherche de cette thèse est qu'il soit possible d'utiliser les concentrations plasmatiques d'un médicament administré par voie pulmonaire (inhalation) pour en déduire l'effet et en optimiser l'utilisation. L'objectif principal consiste à étudier la relation qui existe entre les concentrations plasmatiques et l'effet de la milrinone inhalée suite à son administration chez des patients souffrant d'HP et qui doivent subir une chirurgie cardiaque sous CEC.

Le projet de doctorat comporte deux volets, pharmaceutique et clinique, dont la complémentarité nécessite une étroite collaboration entre les deux milieux. Le volet pharmaceutique porte sur la PK et la détermination de la dose inhalée lors de l'inhalation de la solution intraveineuse de milrinone au moyen des techniques d'administration actuelles. Le volet clinique vise à caractériser la relation PK/PD de la milrinone inhalée à l'aide du biomarqueur PD et à explorer si la réponse à la milrinone peut potentiellement prédire une sortie de CEC difficile chez les patients subissant une chirurgie cardiaque. Ultimement, ces études permettraient de proposer un régime posologique optimal pour la milrinone administrée par inhalation.

## 8.2. Objectifs spécifiques

### 8.2.1. Quantification de la milrinone plasmatique suite à son administration par voie inhalée chez le patient cardiaque

Les méthodes analytiques permettent le dosage des niveaux plasmatiques des médicaments pour lesquels des études pharmacocinétiques sont planifiées. Selon la marge thérapeutique et les propriétés du médicament étudié, les méthodes analytiques peuvent couvrir la quantification d'un large éventail de concentrations pour lesquelles les méthodes se doivent de répondre aux critères de spécificité et de sensibilité requis.

Au cours des études préliminaires, notre laboratoire avait tenté de quantifier les niveaux plasmatiques de la milrinone administrée par voie inhalée chez des patients en chirurgie cardiaque en appliquant les méthodes analytiques utilisées lors d'études

pharmacocinétiques au cours desquelles la milrinone était administrée par voie intraveineuse. La sensibilité de ces méthodes s'étant avérée insuffisante (limite de quantification 5 ng/ml) pour permettre la détection de la milrinone longtemps après son inhalation, nous avons procédé à l'optimisation d'une méthode par chromatographie liquide à haute performance couplée à un détecteur ultraviolet (HPLC-UV) de manière à atteindre les niveaux de spécificité et sensibilité nécessaires à la couverture complète du profil pharmacocinétique.

Les objectifs spécifiques consistaient à optimiser et valider une méthode analytique HPLC-UV:

i. permettant de quantifier simultanément la milrinone et son standard interne (amrinone).
ii. dotée d'une spécificité et d'une sensibilité (limite de quantification <5 ng/ml) suffisantes permettant le dosage de la milrinone administrée par voie inhalée.
iii. exacte (~100%), précise et reproductible (coefficient de variation <15%).

### 8.2.2. Détermination de l'exposition systémique de la milrinone administrée par inhalation chez des patients subissant une chirurgie cardiaque sous circulation extracorporelle

Le côté novateur de la milrinone inhalée en chirurgie cardiaque réside dans la voie d'administration. L'inhalation de la milrinone permettrait de réduire l'exposition systémique (ou la biodisponibilité systémique) et par conséquent, de minimiser l'hypotension sévère souvent associée à l'administration intraveineuse de ce médicament. Toutefois, les niveaux plasmatiques de milrinone suite à l'administration par voie inhalée n'avaient encore jamais été rapportés.

Au cours des dernières années, les spécialistes de l'Institut de Cardiologie de Montréal (ICM) ont systématiquement administré la milrinone par voie inhalée aux patients en chirurgie cardiaque et ce, au moyen de deux techniques de nébulisation; initialement, via un nébuliseur pneumatique « jet » et plus récemment, via un nébuliseur à tamis vibrant « mesh ». Une dose nominale (5 ml) de la solution commercialisée pour administration intraveineuse (1 mg/ml) est normalement déposée dans le nébuliseur afin de viser la posologie recommandée pour l'administration intraveineuse chez l'adulte (50-80 μg/kg).

Or, sachant que les circuits utilisés par les dispositifs d'inhalation ont le potentiel d'entraîner énormément de pertes au niveau du médicament, et ce, à différents niveaux du circuit, nous avons voulu les quantifier afin de déterminer le pourcentage de la dose nominale réellement délivrée aux patients. Ce faisant, ces études nous permettent également d'avoir une meilleure estimation de la biodisponibilité systémique de la milrinone.

Les objectifs spécifiques de nos travaux consistaient à:

i. estimer la dose inhalée *in vitro* lors de l'inhalation de la solution intraveineuse de milrinone en laboratoire au moyen des deux techniques de nébulisation utilisées par l'ICM.
ii. estimer la dose inhalée *in vivo* lors de l'inhalation de la solution intraveineuse de milrinone chez les patients en chirurgie cardiaque au moyen d'un nébuliseur à tamis vibrant « mesh ».
iii. déterminer les niveaux plasmatiques de milrinone suite à son administration par voie inhalée et caractériser sa PK en utilisant l'analyse non compartimentale.

### 8.2.3. Caractérisation de la relation concentration-effet de la milrinone suite à son administration par voie inhalée

Dans le cadre de ce programme de recherche clinique, la milrinone inhalée est utilisée pour prévenir l'hypertension pulmonaire lors du rétablissement de la circulation sanguine chez les patients qui viennent de subir une chirurgie cardiaque nécessitant une CEC. Le volet clinique de cette approche préventive s'inscrit en prolongation des travaux effectués au cours du volet pharmaceutique précédent. Plusieurs études cliniques rapportent que la milrinone inhalée a la capacité de réduire les pressions pulmonaires sans affecter les pressions systémiques chez les patients atteints d'hypertension pulmonaire. Selon certaines études, la milrinone inhalée en prophylaxie (avant la CEC) aurait même le potentiel de prévenir une sortie de CEC difficile, une des conséquences les plus mortelles suite à la chirurgie cardiaque. Malgré les effets bénéfiques rapportés pour la milrinone inhalée, aucune étude n'a encore tenté de caractériser la relation

PK/PD de la milrinone pour cette voie d'administration. Nous avons donc entrepris l'étude de la relation qui existe entre les concentrations plasmatiques et l'effet de la milrinone inhalée dans une sous-population de 28 patients cardiaques de l'ICM.

Les objectifs spécifiques des travaux visaient à:

i. caractériser la relation PK/PD de la milrinone inhalée à l'aide du biomarqueur PD.
ii. explorer si la réponse à la milrinone pouvait potentiellement prédire une sortie de CEC difficile chez les patients lors du rétablissement de la circulation sanguine à la fin de la chirurgie cardiaque (efficacité clinique).

# SECTION II: TRAVAUX DE RECHERCHE

# CHAPITRE 9. Manuscrit n°2: High performance liquid chromatography using UV detection for the quantification of milrinone in plasma: improved sensitivity for inhalation

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**Short title**: Quantification of Inhaled Milrinone in Human Plasma by HPLC using UV detection.

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## 9.1. Abstract

An improved analytical assay was developed and validated for the quantification of milrinone concentrations in patients undergoing cardiac surgery. A solid phase extraction was optimized to isolate milrinone from a plasma matrix followed by HPLC using UV detection. Plasma samples (1 ml) were extracted using a $C_{18}$ solid-phase cartridge. Milrinone was separated on a strong cation exchange analytical column maintained at 23.4°C. The mobile phase consisted of a gradient (10:90 to 45:55), 0.05 M phosphate buffer (pH 3): acetonitrile. Calibration curves were linear in the concentration range of 1.25-320 ng/ml. Mean drug recovery and accuracy were respectively ≥ 96% and ≥ 92%. Intra- and inter-day precisions (CV%) were ≤ 6.7% and ≤ 7.9%, respectively. This method proved to be reliable, specific and accurate. Using different types of column for extraction and separation of milrinone proved to be necessary to achieve the sensitivity and specificity required when milrinone is given by inhalation.

**Keywords**: Assay; Cardiac Surgery; HPLC; Inhalation; Milrinone; Validation.

## 9.2. Introduction

Intravenous milrinone is commonly used to improve ventricular function after cardiac surgery by reducing pulmonary hypertension but is associated with systemic hypotension and increased vasoactive drug requirements.[1] Nebulized vasodilators allow targeted drug delivery, high local concentrations and less systemic hypotension.[2] Indeed, administration of milrinone by inhalation after cardiopulmonary bypass proved to be an alternative mean of reducing pulmonary hypertension while avoiding systemic hypotension.[3,4] More recently, a retrospective analysis suggested a preventive effect when inhaled milrinone is administered before cardiopulmonary bypass.[5] The pharmacokinetics of inhaled milrinone have not been characterized yet and lower plasma concentrations are expected. Although the therapeutic window for intravenous milrinone ranges from 100 to 400 ng/ml,[6] preliminary results in patients given inhaled milrinone before cardiac surgery indicate plasma concentrations below 10 ng/ml after weaning from cardiopulmonary bypass.

Analytical assays used for the determination of milrinone in human plasma have been published suggesting different extraction methods prior to HPLC analysis.[6-8] These assays included either a double liquid-liquid or solid phase extraction (SPE) prior to HPLC using a $C_{18}$ analytical column and UV detection. Pharmacokinetics studies on milrinone most often used the method of Edelson et al.[7] that combined double liquid-liquid extraction with a back extraction. Oddie et al.[8] used SPE while a direct precipitation prior to SPE was added as an additional step by Woolfrey et al..[6] Finally, two other studies proposed a slight modification of Edelson et al.'s method.[9,10] Most assays report a lowest limit of quantification (LLOQ) of 5 ng/ml except two of them where a 1 ng/ml LLOQ is reported.[9,10] Only one report[10] included a detailed validation and when trying to reproduce it, using 1 ml instead of 0.1 ml human plasma, endogenous interferences were such that the lowest sensitivity achieved with our detector was 10 ng/ml.

The aim of the present work was to optimize existing methods in order to reach a higher sensitivity that would be required for the quantification of milrinone given by inhalation in cardiac surgical patients.

## 9.3. Experimental

### 9.3.1. Chemicals and reagents

Milrinone, 1,6-dihydro-2-methyl-6-oxo[3,4'-bipyridine]-5-carbonitrile, was kindly supplied by Sandoz (Boucherville, QC, CAN). Amrinone, the internal standard (I.S.), 5-amino-3,4'-bipyridyl-6(1H)-one, was purchased from Sigma (St. Louis, MO, USA). HPLC grade solvents and reagents (Fisher Scientific, Nepean, ON, CAN) were filtered through a 0.2-µm Type HVLP membrane (Millipore, Billerica, MA, USA) before use. HPLC grade buffer salts were obtained from American Chemical Ltd (Saint-Laurent, QC, CAN). Ultrahigh purified water was obtained from Milli-Q water dispensing system by Millipore Corporation (Billerica, MA, USA).

### 9.3.2. Standard solutions and buffers

Stock solutions (1mg/ml) of pure milrinone standard were prepared in methanol and stored at -20°C. Working solutions (10 µg/ml water) were prepared extemporaneously. A stock solution of amrinone (100 µg/ml) was prepared in methanol and stored at -70°C. The working solution (2.5 µg/ml water) was prepared daily.

The neutralizing solution of 1 M ammonium acetate was prepared in water. The eluting solution of MeOH-HCl (0.02 mol/l) was prepared by adding a concentrated solution of HCL to pre-filtered methanol. Tetrahydrofuran (THF) was added to 0.05M $NaH_2PO_4$ buffer (pH 3) immediately before HPLC analysis to obtain the final THF- $NaH_2PO_4$ buffer (21:916 v/v).

### 9.3.3. Equipment and analytical method

HPLC analysis was performed on a Hewlett Packard 1100 series HPLC system (Wilmington, DE, USA) equipped with a multiple solvent delivery system and a variable wavelength UV/visible detector. Chromatographic separations were carried out on a Spherisorb strong cation exchange (SCX) column (15.0 cm x 4.6mm, 5µm; HiChrom Ltd, Reading, Berkshire, UK) protected by a security cartridge system (Upchurch Scientific Inc., Oak Harbor, WA, USA). Mobile phase consisted of a mixture of ACN: THF-$NaH_2PO_4$ buffer delivered at 1 ml/min using a linear gradient from 10:90 to 45:55

for the first 10 min and maintained at 45: 55 up to 13 min.The column was kept at 23.4°C and run time was set at 13 min with 2 min post-run time. Injection volume was 100 µl and UV detection at 340 nm. Chromatographic peaks were integrated by the ChemStation software (Version A.09.01, Agilent Technologies, Santa-Clara, CA, USA).

### 9.3.4. Plasma calibration curves

Nine concentrations of milrinone ranging from 320 to 1.25 ng/ml (LLOQ) were used to establish plasma calibration curves. Calibration samples were prepared extemporaneously by serial 1:1 dilutions with previously screened blank plasma.

Quality control (QC) samples were prepared by spiking blank plasma with milrinone stock solutions to obtain final concentrations of 7.5 and 100 ng/ml.

Calibration curves were generated from the nine plasma calibration samples by plotting analyte/I.S. peak-height ratio against milrinone concentration. Linearity was assessed using a weighted least square regression ($1/x^2_{nominal}$).

### 9.3.5. Plasma samples preparation

Varian Bond Elut ® $C_{18}$ reversed-phase sorbent (3cc/100 mg) SPE cartridges (Lake Forest, IL, USA) were used to isolate milrinone from plasma samples. To 1 ml of plasma standard/sample, 50 µl of I.S. (125 ng) was added and 1 ml of 1 M ammonium acetate was added to neutralize pH. The solid-phase extraction cartridges were pre-conditioned with methanol (2 x 1ml), water (2 x 1ml) and 1 M ammonium acetate (2 x 1ml). Then, plasma mixture was deposited and slowly aspirated through sorbent bed. Cartridges were then washed with water (3 x 1ml) before recovery of the analyte into a glass tube using 1ml of eluting solution. Extracts were dried at 50°C under a gentle nitrogen stream and reconstituted with 0.2 ml of mobile phase. Each tube was vortexed for 30 sec before transfer of its content into clean, capped 1.5 ml disposable conical polypropylene tubes (Ultident Scientific, Saint-Laurent, QC, CAN). Samples were then centrifuged at 15 850 g for 5 min to remove any particulate matter before injection onto the HPLC column.

## 9.3.6. Clinical application

Milrinone plasma concentration-time profile was determined in a few cardiac patients after a 5 mg dose (Primacor, Sanofi-Synthelabo Canada Inc., Markham, ON, CAN) inhaled over a 15 min period. Informed and written consent was obtained from subjects. Blood samples were obtained before starting inhalation (time zero), at 20, 25, 30 min thereafter, and immediately after cardiopulmonary bypass. Samples were kept in an ice-water bath for less than 10 minutes before centrifuging at 2000 g for 5 minutes. Plasma was immediately flash-frozen on dry ice. Samples were then stored at -70°C until analyzed.

## 9.3.7. Bioanalytical validation

### 9.3.7.1. Specificity

Blank plasma samples from six healthy volunteers were assayed to determine whether endogenous plasma components interfere with the analyte or I.S.

### 9.3.7.2. Linearity

Nine calibration standards (1.25-320 ng/ml) covering the expected clinical range were prepared in plasma.

### 9.3.7.3. Sensitivity

Milrinone at a concentration of 1.25 ng/ml in plasma was extracted and injected on five different days (inter-day). The limit of detection (LOD) of milrinone in the mobile phase was also determined.

### 9.3.7.4. Recovery

Six replicate sets of samples spiked at 7.5 and 100 ng/ml concentrations were prepared. Recovery was assessed by comparing the peak height of milrinone spiked prior and after extraction. Recovery of the I.S. was also determined by comparing the peak heights of six extracted samples with the 100% value determined using in vitro samples.

#### 9.3.7.5. Precision and Accuracy

The intra-assay precision and accuracy were assessed in plasma as follows: 7.5 100 ng/ml QC concentrations were assayed in replicates of 6. All samples were assayed on the same day and their back-calculated concentrations determined from the calibration curve prepared the same day.

Inter-assay precision and accuracy were assessed as follows: 7.5 and 100 ng/ml QC concentrations were assayed in duplicate for each calibration curve. Five calibration curves with their respective QC samples were assayed over five different days.

Precision was expressed as the coefficient of variation (CV, %) and accuracy as the percent bias (%). Accuracy was determined by comparing the calculated concentration of the extracted milrinone plasma standard with the nominal concentration of milrinone.

#### 9.3.7.6. Stability

*Short-term stability.* Bench stability was also verified in duplicates of plasma standards at 7.5 and 100 ng/ml thawed at room temperature and kept at this temperature for 24 h before analysis.

*Freeze-thaw cycles.* Two aliquots of 7.5 and 100 ng/ml were prepared and frozen at –70°C for 24 h. The samples were thawed unassisted at room temperature and analyzed. The samples were refrozen for 24 h under the same conditions. The freeze-thaw cycle was repeated one more time before reanalysis.

*Processed samples stability.* Stability of extracts reconstituted with mobile phase stored in the autosampler at room temperature for 24 h was tested. Results were compared with those obtained for the freshly prepared samples.

*Long-term stability.* Long-term stability of milrinone in plasma stored at -70°C was studied at two plasma concentrations (7.5 and 100 ng/ml). Samples were assayed in duplicate on the day of preparation (day 0), one month and two months later. Long-term stability of aqueous standard solutions was also evaluated at regular intervals. The samples were analyzed every two weeks and were compared with freshly prepared standards.

## 9.4. Results and discussion

### 9.4.1. Optimisation of the method

In preliminaries, milrinone was determined by HPLC using a $C_{18}$ analytical column after direct precipitation of plasma, according to the method described by Lindsay et al.[9] In our conditions, sensitivity of the assay proved to be 10 ng/ml. After analysis of milrinone plasma concentrations in the first patient, it became obvious that this method was not sensitive enough for this route of administration and that plasma constituents often interfered. Several approaches were tried to overcome this problem.

First, Lindsay's procedure was further optimised by using solid-phase extraction on a Bond Elut $C_{18}$ reversed phase sorbent cartridge[8] prior to HPLC instead of direct precipitation[9] for a better sample clean up. Prior neutralization of the plasma and cartridges at pH 7 was necessary to retain milrinone on the cartridge otherwise the extraction recovery was very low. Acidified methanol was used for elution. Therefore, the retention mechanism seems not to be only reverse-phase one but at least partially ionic one.

Then, variations of the mobile phase properties were tested. Retention times of milrinone and I.S. on the $C_{18}$ column proved to depend highly on the pH and ionic strength of the mobile phase. Without any change in the sensitivity, faster elution was achieved by increasing the ionic strength from 0.005 to 0.05 M of $NaH_2PO_4$ in buffer and adjusting the pH from 6.5 to 3. Under the same HPLC conditions, better chromatographic separations were obtained by using a SCX column, and faster elution could be achieved by increasing the ionic strength and the pH of buffer. However, use of a gradient proved to be essential for an optimal elution time of both compounds while avoiding interfering peaks. The run time for this assay is longer than previous methods because interfering peaks were consistently before milrinone. Milrinone peak was monitored at different wavelengths and, in our conditions, 340 nm offered the best sensitivity.

Using these modifications, an excellent recovery and a higher sensitivity (1.25 ng/ml) were achieved.

## 9.4.2. Assay validation

### 9.4.2.1. Specificity

Chromatograms of (a) blank plasma and (b) milrinone spiked plasma used for the calibration curve, and (c) plasma sample obtained in a cardiac patient 5 min after the end of an ultrasonic nebulization of 5 mg milrinone over 15 min are shown in Figure 1. Variation in day-to-day elution times were the followings: Milrinone was generally eluted between 8.1 and 8.5 min and the I.S. was eluted between 11.1 and 11.5 min.

Blank plasma obtained from healthy volunteers (not necessarily in the fasted state) used for each calibration curve and plasma sampled for each patient undergoing cardiac surgery prior to milrinone administration were analyzed and shown to be free of co-extracted endogenous interference. Several drugs are administered for patient's comfort before surgery and during anesthesia induction (lorazepam, morphine, midazolam, sufentanil, pancuronium) as well as during surgical procedure in order to maintain patient's stability. Hence, those drugs are not taken by healthy volunteers, which can explain that disturbances in the blank and patient sample are absent in the spiked sample.

### 9.4.2.2. Linearity

Calibration curves of milrinone in plasma were linear from 1.25 to 320 ng/ml with a mean average correlation coefficient ($r^2$) of 0.9938 (n = 5; range: 0.9908 – 0.9974).

### 9.4.2.3. Sensitivity

Mean inter-assay precision and accuracy for the LLOQ (1.25 ng/ml) were 6% and 98%, respectively (n=5). Intra-assay precision was not evaluated at LLOQ. The LOD in aqueous solution was 0.6 ng/ml.

### 9.4.2.4. Recovery

Mean recoveries of milrinone for the 7.5 and 100 ng/ml concentrations were 96 ± 2.9 % and 104 ± 9 % (n =6), respectively. The mean recovery of I.S. was 100% (CV < 13%, n=6).

#### 9.4.2.5. Intra-assay and inter-assay precision and accuracy

Intra-assay precision study revealed a CV < 7% (n=6) for milrinone concentrations and accuracy ranged from 92 to 99% (Table 1).

Inter-assay precision study revealed a CV < 8% (n=5) for milrinone concentrations and accuracy ranged from 100 to 102% (Table 1).

#### 9.4.2.6. Robustness

Minor variations in the method parameters did not have any effect on experimental results.

#### 9.4.2.7. Stability

*Short-term stability.* Accuracy ranged from 108 to 117%.

*Freeze and thaw cycles.* Accuracy ranged from 92 to 113% after two freeze thaw cycles.

*Processed samples stability.* Accuracy following storage of reconstituted extracts in the autosampler varied between 91-109%.

*Long-term stability.* Milrinone in plasma stored at -70°C was shown to be stable for at least two months. Standard solutions were found stable at least 5 months when refrigerated at -20°C and accuracy was 99.9% (w/w).

### 9.4.3. Application of the method

The level of sensitivity obtained from this analytical method (1.25 ng/ml) proved to be adequate for the characterization of the concentration-time profile of inhaled milrinone in a few cardiac patients after weaning from cardiopulmonary bypass. Pre-dose plasma sample was free of endogenous or drug interferences under the anesthetic procedure. Plasma concentration-time profile of milrinone for this patient was comprised between 19.8 ng/ml (20 min) and 3.6 ng/ml (160 min) (Figure 2).

## 9.5. Conclusion

A highly selective HPLC assay with UV detection has been optimized for determination of milrinone in human plasma. This assay provides a specific and reproducible alternative to currently available methods and, to our best knowledge, offers the level of sensitivity required to study the pharmacokinetics of inhaled milrinone.


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## 9.6. References


[1] B.E. Jaski, M.A. Fifer, R.F. Wright, E. Braunwald, W.S. Colucci, J Clin Invest 75 (1985) 643.

[2] S.L. Katz, I. Adatia, E. Louca, K. Leung, T. Humpl, J.T. Reyes, A.L. Coates, Pediatr Pulmonol 41 (2006) 666.

[3] A. Haraldsson, N. Kieler-Jensen, U. Nathorst-Westfelt, C.H. Bergh, S.E. Ricksten, Chest 114 (1998) 780.

[4] A. Haraldsson s, N. Kieler-Jensen, S.E. Ricksten, Anesth Analg 93 (2001) 1439.

[5] Y. Lamarche, L.P. Perrault, S. Maltais, K. Tetreault, J. Lambert, A.Y. Denault, Eur J Cardiothorac Surg 31 (2007) 1081.

[6] S.G. Woolfrey, J. Hegbrant, H. Thysell, P.A. Fox, D.W. Lendrem, G.F. Lockwood, K. Lasher, J. Rogers, D. Greenslade, J Pharm Pharmacol 47 (1995) 651.

[7] D.S. Baim, A.V. McDowell, J. Cherniles, E.S. Monrad, J.A. Parker, J. Edelson, E. Braunwald, W. Grossman, N Engl J Med 309 (1983) 748.

[8] C.J. Oddie, G.P. Jackman, A. Bobik, J Chromatogr 374 (1986) 209.

[9] C.A. Lindsay, P. Barton, S. Lawless, L. Kitchen, A. Zorka, J. Garcia, A. Kouatli, B. Giroir, J Pediatr 132 (1998) 329.

[10] D.R. Brocks, T.J. Spencer, A. Shayeganpour, J Pharm Pharm Sci 8 (2005) 124.

## 9.7. Tables

**Table 1.** Intra-assay and inter-assay precision and accuracy.

| | | Concentration (ng/ml) | | Precision | Accuracy |
|---|---|---|---|---|---|
| | | Actual | Observed | C.V. | Bias |
| | n | | mean ± S.D. | (%) | (%) |
| Intra-assay | 6 | 7.5 | 6.9 ± 0.5 | 6.7 | -8.1 |
| | 6 | 100 | 98.8 ± 6.3 | 6.4 | -1.2 |
| inter-assay | 5 | 1.25 | 1.2 ± 0.07 | 5.7 | -1.6 |
| | 5 | 7.5 | 7.5 ± 0.6 | 7.9 | 0.1 |
| | 5 | 100 | 102.0 ± 5.0 | 4.9 | 2.0 |

## 9.8. Figures

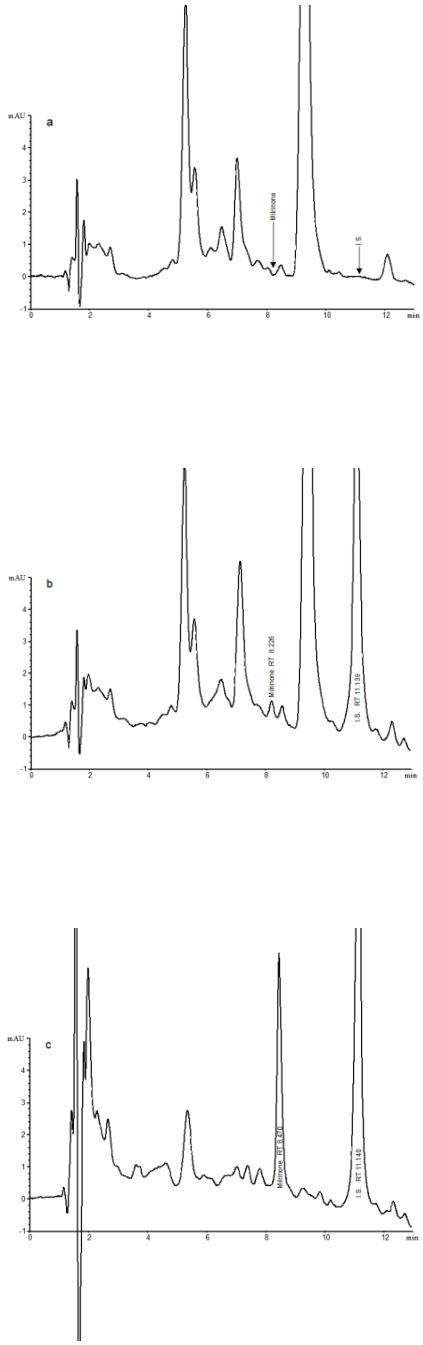


**Figure 1.** Typical HPLC chromatograms after extraction of (a) blank plasma, (b) plasma sample spiked with 2.5 ng/ml of milrinone and I.S., and (c) plasma measured with 42.7 ng/ml of milrinone obtained in a cardiac patient 5 min after the end of 15 min inhalation of 5 mg of milrinone.

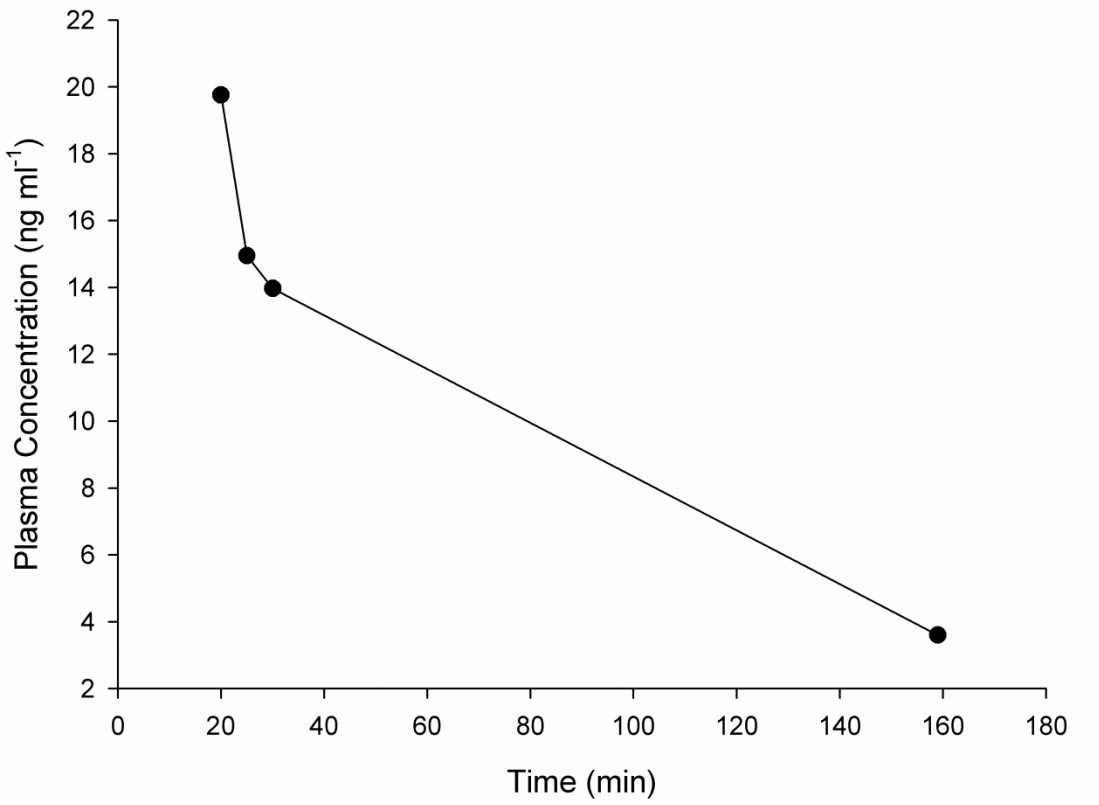


**Figure 2.** Milrinone plasma concentration-time profile in a cardiac patient after inhalation of a 5 mg dose over 15 min. No blood samples were drawn during cardiopulmonary bypass (44 to 159 min).

# CHAPITRE 10. Manuscrit n°3: Inhaled milrinone administered in patients undergoing cardiac surgery. Part I: Inhaled dose and systemic exposure

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**Clinical trial number and registry:** Health Canada CTA (ref: 108851); ClinicalTrials.gov (ref: NCT01725776)

**Short title:** Inhaled dose and systemic exposure after inhaled milrinone

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**Conflicts of interest:** None declared.

**Number of words:** Abstract (268), Introduction (504), Discussion (1361).

## 10.1. Summary

***Background***. Administration of inhaled milrinone before cardiopulmonary bypass has been suggested to have a protective effect during cardiac surgery. However, milrinone pharmacokinetics has never been determined for this route of administration. The objectives of this study carried out in patients undergoing cardiac surgery were twofold: first, to investigate milrinone inhaled dose and early systemic exposure using a jet or a mesh nebulizer and second, to characterize milrinone pharmacokinetic profile.

***Methods***. Pulmonary hypertensive patients scheduled for cardiac surgery were first recruited for a pilot (n=12) and, subsequently, a full-scale (n=15) study where milrinone (5 mg) was administered by inhalation before cardiopulmonary bypass. In the pilot study, *in vitro* experiments were conducted to determine the inhaled dose using a jet or a mesh nebulizer. Blood samples were drawn from patients for the first 15 min post-inhalation to determine early systemic exposure. In the full-scale study, the inhaled dose after mesh nebulization was estimated using *in vivo* dose recovery and 24-h urine collection. After 10h-extensive blood sampling, non-compartmental pharmacokinetic analysis was also carried out.

***Results***. With the mesh nebulizer, mean inhaled dose was threefold higher when compared with the jet nebulizer (46% vs 17%, respectively). Accordingly, early plasma concentrations were significantly higher (2-3 fold) after mesh nebulization in patients. After mesh nebulization and correction for overall losses, mean inhaled dose (1.52 mg; 30%) was confirmed by 24h urinary recovery (1.30 mg; 26%). Using the inhaled dose, milrinone clearance (0.11 $L \cdot h^{-1} \cdot kg^{-1}$) and volume of distribution (0.46 $L \cdot kg^{-1}$) agreed with published data on intravenous milrinone.

***Conclusions***. A threefold higher inhaled dose is observed with the mesh nebulizer. Systemic levels after mesh nebulization remain within milrinone therapeutic range.

**Keywords**: cardiac surgery; inhaled dose; milrinone; nebulizers; patients; pharmacokinetics; plasma; urine.

## 10.2. Introduction

Intravenous milrinone, a positive inotrope and vasodilator, has been extensively used in cardiac surgery for the treatment of pulmonary hypertension (PH) secondary to difficult separation from cardiopulmonary bypass (CPB).[1-4] An important drawback of intravenous milrinone is its association with systemic hypotension.[5-7] To avoid this side effect, inhalation has been proposed as an alternative route of administration for milrinone.[8-10] Drug delivery to the lungs by inhalation allows rapid absorption, high bioavailability and high local concentration.[11] Consequently, the hypothesis that inhaled milrinone administered prior to CPB would have a protective effect in pulmonary hypertensive patients[12,13] by minimizing CPB-related inflammation,[14] preventing pulmonary endothelial dysfunction[15] and facilitating separation from CPB[16] was put forward in the past decade.

Aerosol drug delivery has become one of the major treatments for patients with pulmonary diseases.[17] Nebulizers are commonly used aerosol devices that convert liquid formulations into small droplets to deliver inhaled medications in the treatment of patients with pulmonary diseases.[18] Exception made for two clinical studies,[9,14] jet nebulizers were conventionally used for the administration of milrinone in adult cardiac patients. These nebulizers use a jet of pressurized gas to draw medication from a reservoir and shear the liquid into a wide range of droplet sizes. Recent improvements in nebulizer technologies have led to the development of mesh nebulizers that use a vibrating mesh, powered by either electricity or battery, to force the liquid through multiple apertures and generate aerosol with a high fine particle fraction.[19,20] Because they don't require a secondary airflow and are designed to be compact, portable, and highly efficient for delivering aerosol to the lung with a low residual volume, mesh nebulizers have increased in popularity for delivering inhaled therapeutics, especially for treating mechanically-ventilated patients.[21]

Delivery of nebulized medication to the lungs is a complex process dependent upon a variety of device-related and clinical factors.[18,21-24] *In vitro* testing can provide the end-user with useful information regarding nebulizer performance. The concept of inhaled

dose was originally introduced in 1991 by Smaldone[25] and its quantification under typical clinical conditions has become a well-accepted method for evaluating nebulizer performance.[18,26] This inhaled dose is defined as the amount of medication that is ultimately inhaled by the patient. Thus, device selection and administration technique will highly impact on the pharmacokinetics of inhaled milrinone (e.g., input rate, systemic exposure, etc.) and eventually, response in cardiac patients. While preliminary evidence supports the protective effect of inhaled milrinone administered before CPB in pulmonary hypertensive patients,[12,13] inhaled dose after nebulization, plasma levels and PK parameters have not yet been determined for this route of administration.

This report on inhaled milrinone will present results obtained from a pilot and a full-scale PK study. In the pilot study, after either jet or mesh nebulization, milrinone early systemic exposure was first studied in cardiac patients undergoing CPB while milrinone inhaled dose was determined *in vitro* using a setting similar to that used *in vivo*. For the full-scale study, milrinone definite plasma pharmacokinetics, 24-h urinary excretion as well as *in vivo* inhaled dose were estimated after mesh nebulization in additional patients.

## 10.3. Materials and Methods

### 10.3.1. Patients

After approval by the institutional research ethic committee and with permission from Health Canada (ref: 108851), the study was registered in ClinicalTrials.gov (ref: NCT01725776). Written informed consent was obtained from a total of 27 patients having pre-operative pulmonary hypertension and scheduled for elective cardiac surgery under CPB. First, 12 patients were recruited for the pilot study and subsequently 15 patients were recruited for the full-scale study. Patients were considered having pulmonary hypertension if either one of the following conditions was satisfied before surgery: systolic pulmonary artery pressure (sPAP) >35 mmHg or mean pulmonary artery pressure (mPAP) >25 mmHg.[27] Patients with hemodynamic instability prior to surgery were excluded. Patient characteristics were expressed as mean ± SD.

### 10.3.2. Surgical procedure

In both studies, patients were premedicated with 1-2 mg lorazepam orally 1 hour before surgery and received 0.1 $mg \cdot kg^{-1}$ morphine intramuscularly before entering the operating room where midazolam was given (0.01-0.05 $mg \cdot kg^{-1}$ intravenously) as needed for patient comfort. Usual monitoring was installed, including a 5-lead electrocardiogram, pulse oximeter, peripheral venous line, radial arterial line, 3-lumen catheter, and fast-response thermodilution pulmonary artery catheter. Anaesthesia was induced with 1 $\mu g \cdot kg^{-1}$ sufentanil and 0.04 $mg \cdot kg^{-1}$ midazolam, and muscle relaxation achieved with 0.1 $mg \cdot kg^{-1}$ pancuronium. After tracheal intubation, anaesthesia was maintained with 1 $\mu g \cdot kg^{-1} \cdot h^{-1}$ sufentanil and 0.04 $mg \cdot kg^{-1} \cdot h^{-1}$ midazolam. Cardiopulmonary bypass was instituted using ascending aortic cannulation and bi-caval or double stage cannulation of the right atrium. Intermittent (4:1) blood cardioplegia was administered during CPB; induction and temperatures ranged from 15 to 29ºC. For coronary artery bypass procedures, systemic temperature was allowed to drift to 34ºC, valve and complex procedures to 32-34ºC. Weaning from CPB was undertaken after rewarming to a systemic temperature >36ºC.

## 10.3.3. Drug administration

After induction of anaesthesia and completion of baseline transesophageal echocardiography exam, a dose of 5 mg (50-80 $\mu g \cdot kg^{-1}$) of milrinone (Milrinone Lactate 1 $mg \cdot ml^{-1}$ (base); Pharmaceutical Partners of Canada Inc., Richmond Hill, ON, CAN) was administered by inhalation before initiation of CPB. In the pilot study, 12 patients were randomized to receive milrinone by inhalation using either a jet (Airlife Misty Max 10 Nebulizer; Salter Labs, Arvin, CA, USA) or a mesh nebulizer (Aeroneb Professional Nebulizer System; Aerogen Ltd, Galway, Ireland). Jet nebulization was achieved with a secondary airflow (8 $L \cdot min^{-1}$). In the full-scale study, milrinone was administered using exclusively the mesh nebulizer. The nebulizer was attached to the inspiratory limb of the ventilator Y-connector near the endotracheal tube. Milrinone nominal dose, defined as the total drug dose placed in the nebulizer cup, was nebulized until aerosol production was deemed complete after gentle tapping of the device.

## 10.3.4. Pilot study

### 10.3.4.1. Early systemic exposure

Limited sampling was carried out in cardiac patients randomized to receive a 5 mg dose of milrinone using either a jet or a mesh nebulizer. Arterial blood samples (5 ml) were obtained before starting inhalation (baseline; 0 min) and after the end of inhalation (5, 10, 15 min). Blood samples were kept on ice for a short period of time and centrifuged (3500 rpm; 15 min; 4°C). Plasma was immediately flash-frozen on dry ice and stored at -80°C until analysis. Milrinone plasma concentrations were determined by high-performance liquid chromatography using ultraviolet detection (HPLC-UV).[28] The lower limit of quantification (LLOQ) was 1.25 $ng \cdot ml^{-1}$ with mean intra-assay and inter-assay precisions expressed as coefficients of variation (CV%) less than 8%.

*Data analysis.* Patient characteristics and milrinone concentrations at each time point were expressed as mean ± standard deviation (SD). Between groups (jet vs mesh) comparisons were performed at each sampling time using a 2x2 repeated measures ANOVA. Statistical analysis was carried out with S-PLUS® 8 software (Insightful Corp., Seattle, WA, USA). A *P*-value $<0.05$ was considered statistically significant.

#### 10.3.4.2. In vitro emitted and inhaled dose

The study was designed to closely replicate typical clinical conditions in the operating room. On three different days, experiments that mimic *in vivo* administration of inhaled milrinone to cardiac patients were carried out, for each type of nebulizer, to determine their performance in terms of emitted dose and inhaled dose. The setup consisted of an adult breathing circuit connected to a mechanical ventilator with an expansion chamber (3 L ventilation balloon) placed at the patient interface, *i.e.*, directly connected to the distal end of the endotracheal tube. Representative breathing patterns for our patient population were used: tidal volume of 500 ml, frequency of 12 breaths per minute, minute volume of 6 L per minute and an inspiratory:expiratory ratio of 1:2.[29,30]

Each nebulizer was composed of a cup (main unit) and a T-piece. The jet nebulizer was designed with the T-piece positioned on top of the nebulizer cup (Figure 1A) but *vice versa* for the mesh nebulizer (Figure 1B). Low-resistance collecting filters (Vital Signs Inc., Totowa, NJ, USA) were used to capture milrinone over the duration of the treatment. For each type of nebulizer, two series of experiments were carried out using the same setup but having collecting filters placed at different positions within the breathing circuit. Milrinone administration was as earlier described for patients.

*Setting 1* (Figure 2A) was used to determine the emitted dose, defined as the mass of medication leaving an aerosol generator. For this test, filter A (emitted dose) was placed immediately at the outflow of the nebulizer T-piece.

*Setting 2* (Figure 2B) was used to determine the inhaled dose, defined as the mass of medication that is inhaled by the patient. Drug loss during the expiratory phase (exhaled dose) was also determined. For this test, filter B (inhaled dose) was placed at the distal end of the endotracheal tube, immediately before the expansion chamber (3 L ventilation balloon), and filter C (exhaled dose) was connected to the expiratory limb of the Y-connector.

At the end of inhalation, the nebulizer cup and T-piece were each rinsed with 2 ml of sterilized water to determine the residual dose (i.e., the amount of medication in the residual volume remaining within the nebulizer cup) and drug loss on the nebulizer T-

piece. Milrinone was eluted from filters after immersion in 250 ml buffer solution ($NaH_2PO_4$ 50mM, pH 3) and 10 min ultrasound sonication. Preliminary experiments had confirmed quantitative recovery of milrinone from filters (99.1%, n=4). Samples were stored at -20°C until analysis. Milrinone content was determined by HPLC-UV using a simplified version of the assay used for plasma (see above). Six concentrations of milrinone prepared in buffer solution (20 down to 0.01 µg $ml^{-1}$) were used to establish calibration curves ($r^2$=0.9967, n=6). Each sample was injected twice in the HPLC and the mean value retained for data analysis.

*Data analysis.* Mean total drug mass collected on each component was determined and expressed as percentage (± SD) of nominal dose (5 mg). For each replicate, the total dose recovered was obtained by summing individual recoveries. In *Setting 2* only, drug loss in the Y-connector and the endotracheal tube could not be directly determined due to the difficulty of disconnection without significant spillage. Instead, drug loss in these components was back-calculated by subtracting individual recoveries determined for the other components from the total dose recovered determined in *Setting 1*.

## 10.3.5. Full-scale study

### 10.3.5.1. Pharmacokinetic study

Considering the ease of operation and higher systemic exposure observed after mesh nebulization, a definite PK study was conducted in 15 cardiac patients after inhalation of a 5 mg dose of milrinone using this device only. Serial arterial blood draws (5 ml) were obtained before starting inhalation (baseline; 0 min), during inhalation (2, 5, 10, 15 min), and after the end of inhalation (0, 3, 6, 9, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600 min). Two samples were also obtained 2 min after initiation and separation from CPB. Blood samples were processed as described for the pilot study. Milrinone plasma concentrations were determined by a micromethod using HPLC tandem mass spectrometry detection (HPLC-MS/MS).[31] The LLOQ was 0.3125 ng·$ml^{-1}$ with mean intra-assay and inter-assay precisions (CV%) less than 12%. For each patient, complete urine collection were obtained over a 24h period. The total (conjugated and unconjugated) amount of milrinone excreted was determined using an HPLC-UV

assay.[32] The LLOQ was 31.25 ng·ml$^{-1}$ with mean intra-assay and inter-assay precisions less than 7%.

#### 10.3.5.2. In vivo inhaled dose

After milrinone inhalation using a mesh nebulizer, the residual dose (nebulizer cup) and the exhaled dose (filter C) were determined using the same procedure and analytical assay mentioned under the *in vitro* section above. Since milrinone is almost exclusively excreted in urine (>95%),[33,34] we assumed that the cumulative 24-h urinary excretion corresponded to the *in vivo* inhaled dose ($D_{inh}$).[32] Mean drug masses were expressed as percentage (± SD) of nominal dose (5 mg). The total dose recovered was estimated by summing individual recoveries determined *in vivo* for each patient (including urinary excretion) and mean recovery obtained *in vitro* for components that could not be disconnected during cardiac surgery (i.e., nebulizer T-piece, Y-connector and endotracheal tube). A patients' complete 24-h urine collection is often difficult to obtain in a clinical setting and an alternative approach for the estimation of the inhaled dose was deemed necessary. For this purpose, we also back-calculated the inhaled dose ($D_{inh}$) for each patient by subtracting the total dose recovered from overall drug losses (individual *in vivo* and/or *in vitro* mean values) from the nominal dose. The back-calculated value of $D_{inh}$ was then compared with the cumulative amount of milrinone recovered in urine for that patient using a Student's paired-t test.

*Data analysis.* Parameters including peak concentration (Cmax), peak time (Tmax), elimination rate constant (kel), clearance and volume of distribution expressed as a function of bioavailability (Cl/F, Vd/F) were determined by non-compartmental analysis using WinNonlin® Version 5.3 program (Pharsight Corp., Mountain View, CA, USA).

## 10.4. Results

### 10.4.1. Pilot study

#### 10.4.1.1. Early systemic exposure

Patient characteristics were similar for the mesh and jet nebulizer groups (Table 1). Milrinone mean plasma concentrations and individual concentration-time profiles are presented in Table 2 and Figure 3, respectively. Overall, plasma levels were significantly higher shortly after mesh nebulization.

#### 10.4.1.2. In vitro emitted and inhaled dose

Results from the determination of the emitted dose (*Setting 1*) and the inhaled dose (*Setting 2*) are summarized in Table 3.

For *Setting 1*, mean emitted dose (filter A) was similar for both types of nebulizers (64.0 vs 68.0% for jet and mesh, respectively). However, distribution of drug loss differed within the nebulizer two components. The residual dose was more important with the jet nebulizer while drug loss in the T-piece was greater with the mesh nebulizer. Mean percentage of total dose recovered was 94.7% and 96.5% of nominal dose (5 mg) for the jet and the mesh nebulizers, respectively, with a high degree of consistency (CV% of 2.0% and 2.3%, respectively).

For *Setting 2*, mean inhaled dose (filter B) was almost threefold higher with the mesh (46.4%) compared to the jet (16.6%) nebulizer. Accordingly, a lower exhaled dose (filter C) was observed with the mesh (7.4%) compared to the jet nebulizer (34.1%). Residual doses for both types of nebulizers were similar to those observed after experiments from *Setting 1*. Mean percentage of total dose recovered was 78.6 and 75.1% for the jet and mesh nebulizer, respectively. Consequently, mean back-calculated drug losses in the Y-connector and endotracheal tube were estimated as 16.1 and 21.4% for the jet and mesh nebulizer, respectively.

## 10.4.2. Full-scale study

### 10.4.2.1. Pharmacokinetic study

Patient characteristics and individual milrinone concentration-time profiles for the full-scale study with the mesh nebulizer are presented in Table 1 and Figure 3, respectively. Overall, maximum plasma concentrations (Cmax) ranged between 59 and 189 ng $ml^{-1}$ and were observed between 13 and 27 min (Tmax).

### 10.4.2.2. In vivo inhaled dose

Mean percentages of dose recovered after administration of inhaled milrinone in patients are presented in the lower panel of Table 3. Mean residual dose in the nebulizer was very similar to that measured *in vitro* (3.5% vs 3.1%, respectively). However, mean exhaled dose (filter C) was almost fourfold greater in patients (26.4%) compared to *in vitro* (*Setting 2*, 7.4%). Mean percentage of total dose recovered *in vivo* was estimated as 95.6%. In patients (n=15), mean cumulative amount of milrinone excreted in urine over a 24-h period represented 26.1% (1.30 mg) of nominal dose (5 mg) while the mean back-calculated value of $D_{inh}$ was 30.5% (1.52 mg). As corresponding values did not differ ($P$=0.112), this approach using back-calculated value of $D_{inh}$ was considered valid for milrinone and used for PK analysis.

Results obtained after performing non-compartmental data analysis are presented in Table 4.

## 10.5. Discussion

This is the first report on milrinone systemic exposure and pharmacokinetics after inhalation in cardiac patients. Milrinone was rapidly absorbed through the lungs. In agreement with their respective inhaled dose, early plasma concentrations were about threefold higher with the mesh compared to the jet nebulizer. After mesh nebulization of milrinone in patients, approximately one fourth of the dose only was inhaled, a finding confirmed by milrinone dose recovery in urine. After inhalation, milrinone PK parameters were in agreement with those previously reported after intravenous administration, suggesting complete bioavailability.

A major benefit attained with nebulizers is their property of transforming drug solutions into an aerosol mist that can be easily inhaled into patients' lower respiratory tract. However, their performance varies between different types of nebulizers[24] and is affected by fill volume, flow and nebulizer brand.[22] Furthermore, factors governing aerosol delivery in mechanically-ventilated patients differ from those in spontaneous breathing patients.[21,24] *In vitro* studies were therefore required for quantitative assessment of the inhaled dose and proper interpretation of PK data.

*In vitro* experiments (*Setting 1*) showed that both jet and mesh nebulizers generated similar emitted doses (Table 3). However, the residual dose in the nebulizer cup was significantly lower with the mesh nebulizer and consistent throughout experiments. Indeed, high stability and minimal residual volume are two well-known properties for this type of nebulizer.[19,21,24] It is worth mentioning that, although drug losses within the nebulizer components were distributed differently for both types of nebulizers, approximately 30% of milrinone nominal dose remained trapped within the nebulizer components in both settings (*Setting 1* and *2*) for both types of nebulizers, which also suggests that the presence of filters placed at different positions did not influence devices performance. In addition, results from *Setting 2* showed that milrinone inhaled and exhaled doses together accounted for 50%, for both types of nebulizers. Accordingly, it was estimated that the remaining drug losses in the ventilator circuit, including the Y-connector and endotracheal tube, would be approximately 20% of the nominal dose.

Considering efficiency in drug delivery, *in vitro* inhaled dose was less than 20% of milrinone nominal dose with the jet nebulizer and threefold higher (almost 50%) with the mesh nebulizer. Similar findings were reported by others testing the same brands of nebulizers.[19,23,24] In mechanically ventilated patients, 2-3 fold more inhaled drug was also obtained with the same model of mesh nebulizer compared to a standard (jet) small-volume nebulizer placed in the same position.[19,20,35] From our *in vitro* experiments, the poor efficiency in delivering the inhaled dose observed with the jet nebulizer coincided with a proportionally higher exhaled dose.

In the pilot study, milrinone systemic exposure was affected by the type of nebulizer and resulted in plasma levels 2-3 fold greater shortly after mesh nebulization, which was in agreement with their respective inhaled dose during *in vitro* experiments. Therefore, in order to provide a higher efficiency for aerosol delivery to the lung, the full-scale study was conducted with the mesh nebulizer only. Indeed, target steady-state plasma concentrations after IV infusion of milrinone in cardiac patients range between 150 and 250 ng $ml^{-1}$.[36] After administration of a 5 mg dose of milrinone using either a mesh or jet nebulizer, in both the pilot and full-scale studies, plasma levels observed in our patients were below 190 ng $ml^{-1}$ and well below those reported after a 50 μg $kg^{-1}$ IV bolus dose of milrinone (over 600 ng $ml^{-1}$).[37]

In the full-scale study as well as during *in vitro* experiments, milrinone inhaled and exhaled doses together represented approximately 50% of the nominal dose after mesh nebulization. However, an almost fourfold increase in the exhaled dose was observed *in vivo* compared to *in vitro*, which translates in a reduced *in vivo* inhaled dose (26% recovered in urine) compared to *in vitro* (46%). These *in vitro*/*in vivo* discrepancies can be explained by several variables. Efficiency of a nebulizer system for aerosol delivery in mechanically-ventilated patients is highly influenced by three main categories of factors that are: circuit-related, ventilator-related or device-related [21,24] In our opinion, humidity would be the major contributing factor amongst circuit-related factors. In contrast to *in vitro* conditions, gas mixture is heated and humidified to prevent drying of the airway mucosa in patients. This humidification leads to an increased loss of aerosol in the circuit. Several *in vitro* studies have shown up to 50% (44% in our case) reduction

in aerosol delivery with heated/humidified circuit. [38-40] Thus, higher drug doses are often suggested to offset the reduced efficiency of nebulizers in mechanically-ventilated patients.[41-44] Ventilator-related factors may also result in significant differences in aerosol drug delivery. A tidal volume of 500 ml or more in adults,[45] longer inspiratory time and slower inspiratory flows improved aerosol delivery in ventilator-dependent patients.[45,46] Although these parameters of the ventilator breath were fixed during our *in vitro* experiments, they were allowed to vary in patients according to their weight and pulmonary conditions, which in turn contributed to the higher variability observed *in vivo* compared to *in vitro*. Finally, studies using *in vitro* simulated models of mechanical ventilation, scintigraphy with radiolabeled aerosols and/or PK clinical studies have also been carried out to optimize techniques of administration by various devices.[47] For a given nebulizer, discrepancies between values obtained with bench models versus those obtained by gamma scintigraphy were observed.[40]

Pharmacokinetic methods based on urinary data have been proposed as a means of determining the extent of the relative deposition of drugs to the lungs following inhalation.[48] After nebulization, pulmonary bioavailability of albuterol (90% excreted in urine) has also been reported using urinary excretion.[47,49,50] However, it is difficult to rely on a 24-h urine collection in a hospital setting, and we therefore examined an alternate method for the estimation of the inhaled dose ($D_{inh}$) required for PK analyses. Mean back-calculated $D_{inh}$ was not significantly different from than that obtained using complete urinary collections. These results also suggest that milrinone pulmonary bioavailability would be nearly complete after mesh nebulization. Our finding is in agreement with a high efficiency of drug delivery to the lower airways and lung periphery that results, in turn, from a high fine-particle fraction (FPF), i.e., percentage of aerosol within a targeted mass median aerodynamic diameter between 1 and 5 μm,[51,52] generated with the mesh nebulizer.[19] In our patients, milrinone was rapidly absorbed into systemic circulation during inhalation. Peak plasma concentrations (Cmax) coincided with the end of inhalation, although the time required for complete nebulization of 5 ml of milrinone solution was quite variable from one patient to another (range: 10-26 min).

Therefore, close monitoring of the end of nebulization proved essential for a good characterization of the plasma concentration-time profile.

In the full-scale study, non-compartmental analysis was carried out to determine milrinone definite PK parameters (Table 4). Our values are quite similar to those reported after an intravenous bolus dose and/or infusion of milrinone in cardiac patients. Milrinone elimination half-life ($T_{1/2}$: 2.6 h) was is the range of values reported (2.3-2.6 h).[53] Most PK studies carried out in cardiac patients,[33,53-55] reported ranges for milrinone clearance (0.11-16 $L \cdot h^{-1} \cdot kg^{-1}$) and volume of distribution (0.30-0.47 $L \cdot kg^{-1}$) that included our mean parameter estimates after inhalation (Cl/F: 0.11 $L \cdot h^{-1} \cdot kg^{-1}$ and Vd/F: 0.46 $L \cdot kg^{-1}$). This good agreement with published PK data indicates that our estimation of the inhaled dose ($D_{inh}$) in patients by back-calculation is probably accurate, or at least very close to reality.

The main limitation of our studies, which constitutes the purpose of additional ongoing studies, consists of the absence of a full characterization of *in vitro* aerosol particle size distribution after jet or mesh nebulization in conditions similar to those prevailing in mechanically-ventilated patients. These studies would provide meaningful insight on the *in vivo* pulmonary deposition pattern for inhaled milrinone.

In conclusion, this is the first report characterizing the inhaled dose and pharmacokinetics of milrinone after inhalation. A low systemic exposure is observed with both jet and mesh nebulizers. However, the mesh nebulizer provides a higher efficiency for aerosol drug delivery in mechanically-ventilated patients resulting in a threefold increased inhaled dose and, consequently, a proportionally higher systemic exposure compared to the conventional jet nebulizer. These results warrant further investigations on the concentration-effect relationship of inhaled milrinone in cardiac patients (refer to manuscript Part II).

## 10.6. References


1. Doolan LA, Jones EF, Kalman J, Buxton BF, Tonkin AM: A placebo-controlled trial verifying the efficacy of milrinone in weaning high-risk patients from cardiopulmonary bypass. J Cardiothorac Vasc Anesth 1997; 11: 37-41

2. Solina A, Papp D, Ginsberg S, Krause T, Grubb W, Scholz P, Pena LL, Cody R: A comparison of inhaled nitric oxide and milrinone for the treatment of pulmonary hypertension in adult cardiac surgery patients. J Cardiothorac Vasc Anesth 2000; 14: 12-7

3. Feneck RO, Sherry KM, Withington PS, Oduro-Dominah A: Comparison of the hemodynamic effects of milrinone with dobutamine in patients after cardiac surgery. J Cardiothorac Vasc Anesth 2001; 15: 306-15

4. Solina AR, Ginsberg SH, Papp D, Grubb WR, Scholz PM, Pantin EJ, Cody RP, Krause TJ: Dose response to nitric oxide in adult cardiac surgery patients. J Clin Anesth 2001; 13: 281-6

5. Jaski BE, Fifer MA, Wright RF, Braunwald E, Colucci WS: Positive inotropic and vasodilator actions of milrinone in patients with severe congestive heart failure. Dose-response relationships and comparison to nitroprusside. J Clin Invest 1985; 75: 643-9

6. Cuffe MS, Califf RM, Adams KF, Jr., Benza R, Bourge R, Colucci WS, Massie BM, O'Connor CM, Pina I, Quigg R, Silver MA, Gheorghiade M: Short-term intravenous milrinone for acute exacerbation of chronic heart failure: a randomized controlled trial. Jama 2002; 287: 1541-7

7. Couture P, Denault AY, Pellerin M, Tardif JC: Milrinone enhances systolic, but not diastolic function during coronary artery bypass grafting surgery. Can J Anaesth 2007; 54: 509-22

8. Haraldsson A, Kieler-Jensen N, Ricksten SE: The additive pulmonary vasodilatory effects of inhaled prostacyclin and inhaled milrinone in postcardiac surgical patients with pulmonary hypertension. Anesth Analg 2001; 93: 1439-45

9. Sablotzki A, Starzmann W, Scheubel R, Grond S, Czeslick EG: Selective pulmonary vasodilation with inhaled aerosolized milrinone in heart transplant candidates. Can J Anaesth 2005; 52: 1076-82

10. Wang H, Gong M, Zhou B, Dai A: Comparison of inhaled and intravenous milrinone in patients with pulmonary hypertension undergoing mitral valve surgery. Adv Ther 2009; 26: 462-8

11. Patton JS, Byron PR: Inhaling medicines: delivering drugs to the body through the lungs. Nat Rev Drug Discov 2007; 6: 67-74

12. Hegazy N, Elhenawy A: Comparison of Hemodynamic Effects of Inhaled Milrinone and Inhaled Prostacyclin after Adult Cardiac Surgery. J Appl Sci Res 2010; 6: 38-44

13. Denault A, Haddad F, Lamarche Y, Nguyen A, Varin F: Pilot randomized controlled trial of inhaled milrinone in high-risk cardiac surgical patients. Surgery Curr Res 2014; 4: 192

14. Gong M, Lin XZ, Lu GT, Zheng LJ: Preoperative inhalation of milrinone attenuates inflammation in patients undergoing cardiac surgery with cardiopulmonary bypass. Med Princ Pract 2012; 21: 30-5

15. Lamarche Y, Malo O, Thorin E, Denault A, Carrier M, Roy J, Perrault LP: Inhaled but not intravenous milrinone prevents pulmonary endothelial dysfunction after cardiopulmonary bypass. J Thorac Cardiovasc Surg 2005; 130: 83-92

16. Lamarche Y, Perrault LP, Maltais S, Tetreault K, Lambert J, Denault AY: Preliminary experience with inhaled milrinone in cardiac surgery. Eur J Cardiothorac Surg 2007; 31: 1081-7

17. Terzano C, Allegra L: Importance of drug delivery system in steroid aerosol therapy via nebulizer. Pulm Pharmacol Ther 2002; 15: 449-54

18. Ari A: Jet, Ultrasonic, and Mesh Nebulizers An Evaluation of Nebulizers for Better Clinical Outcomes. Euras J Pulm 2014; 16: 1-7

19. Dhand R: Nebulizers that use a vibrating mesh or plate with multiple apertures to generate aerosol. Respir Care 2002; 47: 1406-16; discussion 1416-8

20. Vecellio L: The mesh nebuliser: a recent technical innovation for aerosol delivery. Breathe 2006; 2: 253-260

21. Dhand R: Aerosol delivery during mechanical ventilation: from basic techniques to new devices. J Aerosol Med Pulm Drug Deliv 2008; 21: 45-60

22. Hess D, Fisher D, Williams P, Pooler S, Kacmarek RM: Medication nebulizer performance. Effects of diluent volume, nebulizer flow, and nebulizer brand. Chest 1996; 110: 498-505

23. Rau JL, Ari A, Restrepo RD: Performance comparison of nebulizer designs: constant-output, breath-enhanced, and dosimetric. Respir Care 2004; 49: 174-9

24. Ari A, Atalay OT, Harwood R, Sheard MM, Aljamhan EA, Fink JB: Influence of nebulizer type, position, and bias flow on aerosol drug delivery in simulated pediatric and adult lung models during mechanical ventilation. Respir Care 2010; 55: 845-51

25. Smaldone GC: Drug delivery via aerosol systems: concept of "aerosol inhaled". J Aerosol Med 1991; 4: 229-35

26. Dolovich MB: Assessing nebulizer performance. Respir Care 2002; 47: 1290-301; discussion 1301-4

27. Moraes D, Loscalzo J: Pulmonary hypertension: newer concepts in diagnosis and management. Clin Cardiol 1997; 20: 676-82

28. Nguyen AQN, Theoret Y, Chen C, Denault A, Varin F: High performance liquid chromatography using UV detection for the quantification of milrinone in plasma: improved sensitivity for inhalation. J Chromatogr B Analyt Technol Biomed Life Sci 2009; 877: 657-60

29. Williams L, Fletcher GC, Daniel M, Kinsella J: A simple in vitro method for the evaluation of an ultrasonic nebulizer for drug delivery to intubated, ventilated patients and the effect of nebulizer and ventilator settings on the uptake of fluid from the nebulizer chamber. Eur J Anaesthesiol 1999; 16: 479-84

30. Dennis JH: Standardization issues: in vitro assessment of nebulizer performance. Respir Care 2002; 47: 1445-55; discussion 1455-8

31. Gavra P, Nguyen AQ, Theoret Y, Litalien C, Denault AY, Varin F: A specific and sensitive HPLC-MS/MS micromethod for milrinone plasma levels determination after inhalation in cardiac patients. Ther Drug Monit 2014; 36: 663-8

32. Gavra P, Nguyen AQ, Beauregard N, Denault AY, Varin F: High-performance liquid chromatography assay using ultraviolet detection for urinary quantification of milrinone concentrations in cardiac surgery patients undergoing cardiopulmonary bypass. Biomed Chromatogr 2014; 28: 1084-9

33. Stroshane RM, Koss RF, Biddlecome CE, Luczkowec C, Edelson J: Oral and intravenous pharmacokinetics of milrinone in human volunteers. J Pharm Sci 1984; 73: 1438-41

34. Larsson R, Liedholm H, Andersson KE, Keane MA, Henry G: Pharmacokinetics and effects on blood pressure of a single oral dose of milrinone in healthy subjects and in patients with renal impairment. Eur J Clin Pharmacol 1986; 29: 549-53

35. Abdelrahim ME, Plant P, Chrystyn H: In-vitro characterisation of the nebulised dose during non-invasive ventilation. J Pharm Pharmacol 2010; 62: 966-72

36. Woolfrey SG, Hegbrant J, Thysell H, Fox PA, Lendrem DW, Lockwood GF, Lasher K, Rogers J, Greenslade D: Dose regimen adjustment for milrinone in congestive heart failure patients with moderate and severe renal failure. J Pharm Pharmacol 1995; 47: 651-5

37. Butterworth JF, Hines RL, Royster RL, James RL: A pharmacokinetic and pharmacodynamic evaluation of milrinone in adults undergoing cardiac surgery. Anesth Analg 1995; 81: 783-92

38. Dhand R, Tobin MJ: Inhaled bronchodilator therapy in mechanically ventilated patients. Am J Respir Crit Care Med 1997; 156: 3-10

39. Dhand R, Mercier E: Effective inhaled drug administration to mechanically ventilated patients. Expert Opin Drug Deliv 2007; 4: 47-61

40. Miller DD, Amin MM, Palmer LB, Shah AR, Smaldone GC: Aerosol delivery and modern mechanical ventilation: in vitro/in vivo evaluation. Am J Respir Crit Care Med 2003; 168: 1205-9

41. Dhand R, Jubran A, Tobin MJ: Bronchodilator delivery by metered-dose inhaler in ventilator-supported patients. Am J Respir Crit Care Med 1995; 151: 1827-33

42. Dhand R, Duarte AG, Jubran A, Jenne JW, Fink JB, Fahey PJ, Tobin MJ: Dose-response to bronchodilator delivered by metered-dose inhaler in ventilator-supported patients. Am J Respir Crit Care Med 1996; 154: 388-93

43. Duarte AG, Momii K, Bidani A: Bronchodilator therapy with metered-dose inhaler and spacer versus nebulizer in mechanically ventilated patients: comparison of magnitude and duration of response. Respir Care 2000; 45: 817-23

44. Mouloudi E, Maliotakis C, Kondili E, Kafetzakis A, Georgopoulos D: Duration of salbutamol-induced bronchodilation delivered by metered-dose inhaler in mechanically ventilated COPD patients. Monaldi Arch Chest Dis 2001; 56: 189-94

45. Fink JB, Dhand R, Duarte AG, Jenne JW, Tobin MJ: Aerosol delivery from a metered-dose inhaler during mechanical ventilation. An in vitro model. Am J Respir Crit Care Med 1996; 154: 382-7

46. Dolovich MA: Influence of inspiratory flow rate, particle size, and airway caliber on aerosolized drug delivery to the lung. Respir Care 2000; 45: 597-608

47. Marik P, Hogan J, Krikorian J: A comparison of bronchodilator therapy delivered by nebulization and metered-dose inhaler in mechanically ventilated patients. Chest 1999; 115: 1653-7

48. Chrystyn H: Methods to identify drug deposition in the lungs following inhalation. Br J Clin Pharmacol 2001; 51: 289-99

49. Hindle M, Chrystyn H: Determination of the relative bioavailability of salbutamol to the lung following inhalation. Br J Clin Pharmacol 1992; 34: 311-5

50. Silkstone VL, Corlett SA, Chrystyn H: Determination of the relative bioavailability of salbutamol to the lungs and systemic circulation following nebulization. Br J Clin Pharmacol 2002; 54: 115-9

51. Byron PR: Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. J Pharm Sci 1986; 75: 433-8

52. Laube BL: In vivo measurements of aerosol dose and distribution: clinical relevance. J Aerosol Med 1996; 9 Suppl 1: S77-91

53. Edelson J, Stroshane R, Benziger DP, Cody R, Benotti J, Hood WB, Jr., Chatterjee K, Luczkowec C, Krebs C, Schwartz R: Pharmacokinetics of the bipyridines amrinone and milrinone. Circulation 1986; 73: III145-52

54. Bailey JM, Levy JH, Kikura M, Szlam F, Hug CC, Jr.: Pharmacokinetics of intravenous milrinone in patients undergoing cardiac surgery. Anesthesiology 1994; 81: 616-22

55. Das PA, Skoyles JR, Sherry KM, Peacock JE, Fox PA, Woolfrey SG: Disposition of milrinone in patients after cardiac surgery. Br J Anaesth 1994; 72: 426-9

## 10.7. Tables

**Table 1.** Patient characteristics. All values are mean ± SD.

| | Type of nebulizer | n | Gender (F : M) | Age (yr) | Weight (kg) |
|---|---|---|---|---|---|
| Pilot study | Jet | 6 | 5 : 1 | 65 ± 9 | 76 ± 20 |
| | Mesh | 6 | 4 : 2 | 74 ± 8 | 73 ± 23 |
| Full scale study | Mesh | 15 | 4 : 11 | 68 ± 10 | 72 ± 11 |

**Table 2. Pilot study.** Milrinone plasma concentrations in cardiac surgical patients shortly after jet (n=6) or mesh nebulization (n=6). All values are mean ± SD.

| Post-inhalation sampling time (min) | Type of nebulizer: Jet n | Jet Concentration ($ng \cdot ml^{-1}$) | Mesh n | Mesh Concentration ($ng \cdot ml^{-1}$) | *P*-value |
|---|---|---|---|---|---|
| 0 | 1 | 16.1 ± N/A | 3 | 38.5 ± 17.7 | N/A |
| 5 | 6 | 17.7 ± 7.0 | 6 | 53.6 ± 20.0 | 0.002 |
| 10 | 5 | 12.7 ± 3.4 | 6 | 42.9 ± 15.5 | 0.002 |
| 15 | 6 | 14.0 ± 6.8 | 6 | 34.4 ± 10.6 | 0.003 |

**Table 3.** *In vitro* and *in vivo* experiments for milrinone dose recovery. All values are mean ± SD and expressed as percentage (%) of nominal dose (5 mg). *Back-calculated data. **Data obtained from *in vitro* experiments (see Materials and Methods for details).

| | | | Type of nebulizer | | | |
|---|---|---|---|---|---|---|
| | | | Jet | | Mesh | |
| | | | n | (%) | n | (%) |
| **Pilot study** *In vitro* | *Setting 1* | Emitted dose (filter A) | 3 | 64.0 ± 0.5 | 3 | 68.0 ± 5.9 |
| | | Residual dose (nebulizer cup) | 3 | 29.7 ± 1.6 | 3 | 3.1 ± 0.2 |
| | | Drug loss (nebulizer T-piece) | 2 | 1.0 ± 0.2 | 3 | 25.4 ± 3.9 |
| | | **Total dose recovered** | | **94.7 ± 2.0** | | **96.5 ± 2.3** |
| | *Setting 2* | Inhaled dose (filter B) | 3 | 16.6 ± 1.7 | 3 | 46.4 ± 9.6 |
| | | Exhaled dose (filter C) | 3 | 34.1 ± 4.8 | 3 | 7.4 ± 0.2 |
| | | Residual dose (nebulizer cup) | 3 | 27.0 ± 0.6 | 3 | 3.1 ± 0.2 |
| | | Drug loss (nebulizer T-piece) | 3 | 0.9 ± 0.1 | 3 | 18.2 ± 4.4 |
| | | Drug loss (Y-connector + endotracheal tube)* | 3 | 16.1 ± 5.8 | 3 | 21.4 ± 5.7 |
| | | **Total dose recovered (from *Setting 1*)** | | **94.7** | | **96.5** |
| **Full scale study** *In vivo* | *Setting 2* | Inhaled dose (24-h urine collection) | | | 15 | 26.1 ± 7.7 |
| | | Exhaled dose (filter C) | | | 14 | 26.4 ± 6.5 |
| | | Residual dose (nebulizer cup) | | | 14 | 3.5 ± 1.3 |
| | | Drug loss (nebulizer T-piece)** | | | | 18.2 |
| | | Drug loss (Y-connector + endotracheal tube)** | | | | 21.4 |
| | | **Total dose recovered** | | | | **95.6 ± 10.7** |

**Table 4. Full-scale study.** Milrinone PK parameters after non-compartmental analysis. All values are mean ± SD, n=15. $D_{inhaled}$, inhaled dose; Tmax, peak time; Cmax, peak concentration; kel, elimination rate constant; Cl, clearance; F, bioavailability; Vd, volume of distribution. *Inhaled doses calculated ccording to *in vivo* and *in vitro* results (see Materials and Methods for details).

| $D_{inhaled}$* (mg) | Tmax (min) | Cmax ($ng \cdot ml^{-1}$) | kel ($min^{-1}$) | Cl/F ($L \cdot h^{-1} \cdot kg^{-1}$) | Vd/F ($L \cdot kg^{-1}$) |
|---|---|---|---|---|---|
| 1.52 ± 0.32 | 20 ± 4 | 125 ± 38 | 0.0045 ± 0.0017 | 0.11 ± 0.06 | 0.46 ± 0.29 |

## 10.8. Figures

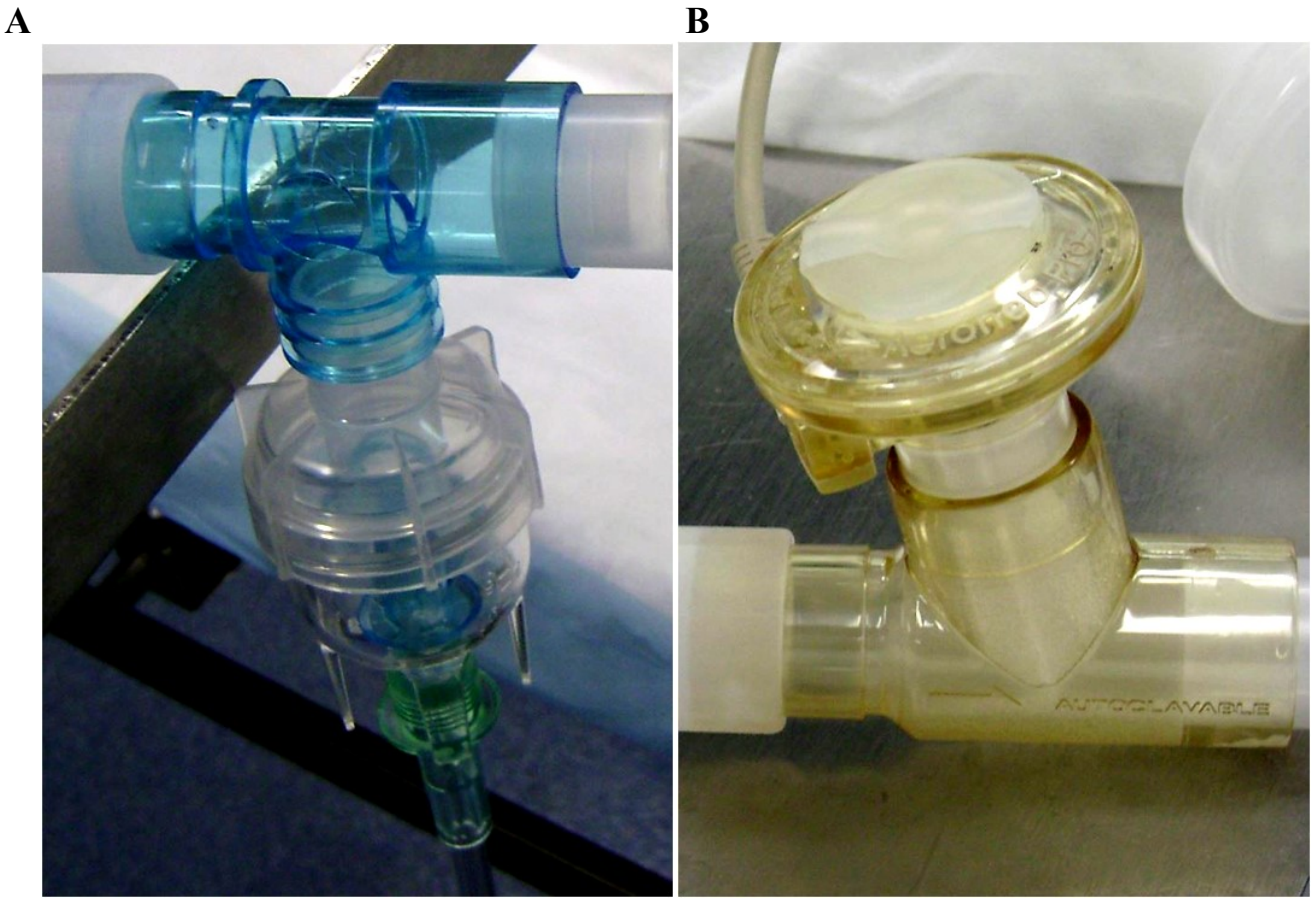


**Figure 1.** Jet nebulizer (Airlife Misty Max 10 Nebulizer; Salter Labs, Arvin, CA, USA) (**A**). Mesh nebulizer (Aeroneb Professional Nebulizer System; Aerogen Ltd, Galway, Ireland) (**B**).

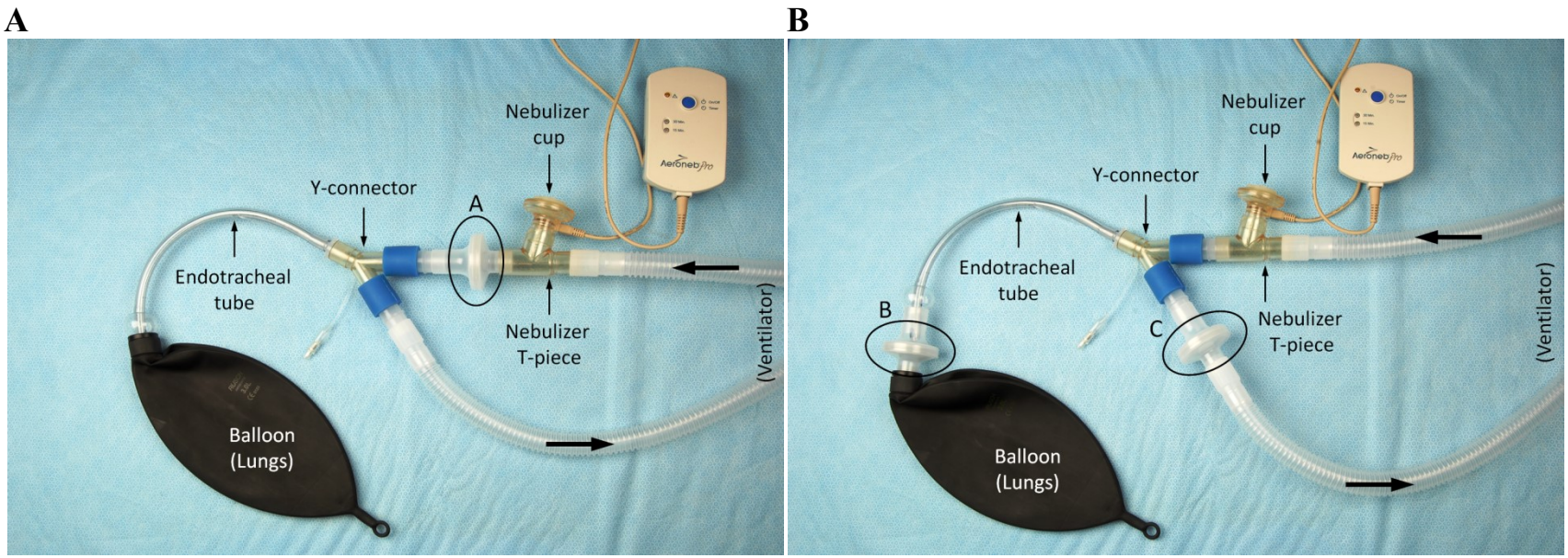


**Figure 2.** *In vitro* settings for estimation of milrinone dose recovery (mesh nebulizer displayed). *Setting 1* (**A**) was used to determine the emitted dose (filter A). *Setting 2* (**B**) was used to determine the inhaled dose (filter B) and exhaled dose (filter C). Residual doses in nebulizer cup and T-piece were also measured.

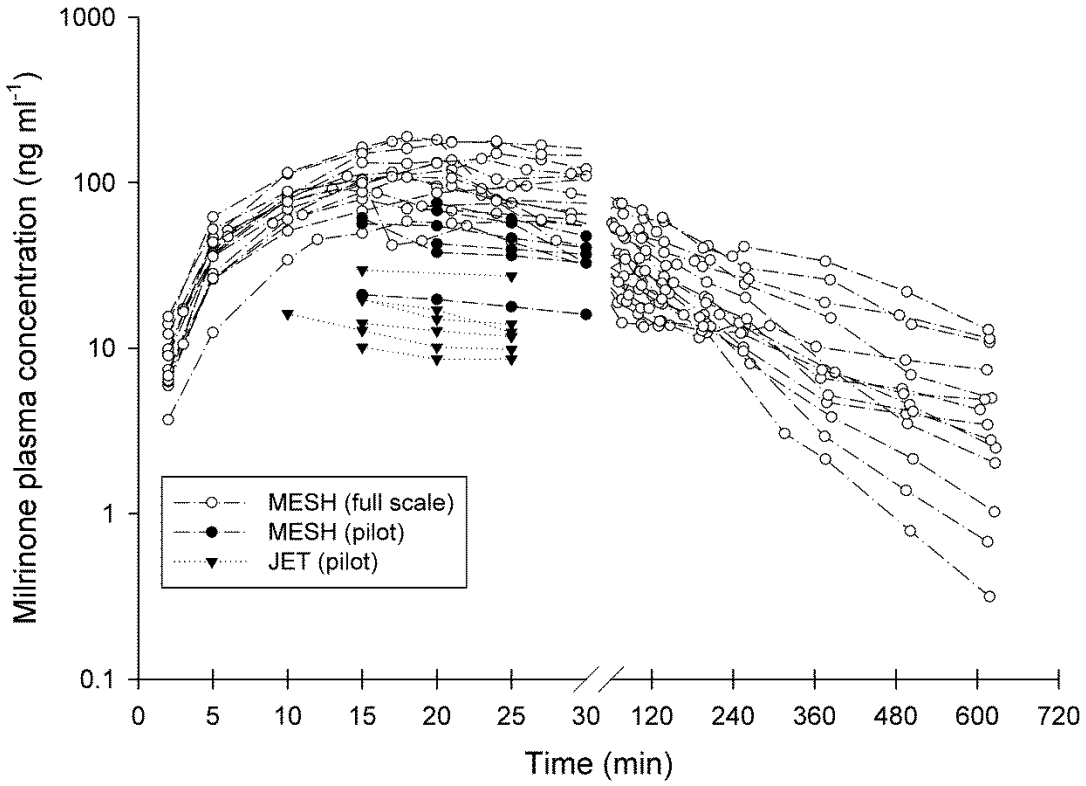


**Figure 3.** Milrinone plasma concentration-time profiles for 27 cardiac patients after limited (pilot study, n=12) and extensive (full-scale study, n=15) sampling following administration of a 5 mg dose using a jet or mesh nebulizer.

# CHAPITRE 11. Manuscrit n°4: Inhaled milrinone administered in patients undergoing cardiac surgery. Part II: Pharmacokinetic/Pharmacodynamic exploration of a biomarker for the preventive effect on pulmonary hypertension associated with cardiopulmonary bypass

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**Short title:** Inhaled milrinone pharmacokinetics and pharmacodynamics

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**Conflicts of interest:** None declared.

**Number of words:** Abstract (250), Introduction (277), Discussion (1601).

## 11.1. Summary

***Background.*** Inhaled milrinone before cardiopulmonary bypass (CPB) has been proposed to prevent difficult separation from CPB (DSB) in cardiac patients with pulmonary hypertension. Mean arterial pressure to mean pulmonary arterial pressure ratio (mAP/mPAP) was identified as the best predictor of perioperative complications. We investigated the pharmacokinetic/pharmacodynamic (PK/PD) relationship of inhaled milrinone in these patients using this ratio (R) as a PD marker.

***Methods.*** Before initiation of CPB in 28 consenting patients, milrinone (5 mg) was nebulized and plasma concentrations measured up to 10 hours after inhalation. Baseline ($R_0$), peak ($R_{max}$), and post-CPB ($R_{post\text{-}CPB}$) ratios as well as magnitude of peak response ($\Delta_{Rmax\text{-}R0}$) were measured. Milrinone concentration-effect relationship was characterized during inhalation by correlating individual area under effect-time (AUEC) and area under plasma concentration-time (AUC) curves. Potential relationships between PD drivers and the clinical endpoint DSB were explored.

***Results.*** Milrinone peak concentrations (41-189 ng $ml^{-1}$) and $\Delta_{Rmax\text{-}R0}$ (-0.12-1.5; -5-65%) were observed at the end of inhalation (10-30 min). Mean PK estimates agreed with intravenous milrinone published data. Paired comparisons between $R_0$ and $R_{max}$ or $R_{post\text{-}CPB}$ were statistically different ($P$ <0.001) and $\Delta_{Rmax\text{-}R0}$ found associated with absence of DSB ($P$=0.009). During inhalation, individual AUEC directly correlated with AUC ($P$=0.045); significance increased after exclusion of non-responders ($P$=0.024). Individual AUEC correlated with $\Delta_{Rmax\text{-}R0}$ ($P$=0.001). Both $\Delta_{Rmax\text{-}R0}$ ($P$=0.009) and CPB duration ($P$ <0.001) were identified as predictors of DSB.

***Conclusions.*** After inhaled milrinone, mAP/mPAP ratio appears a promising PD marker. Both magnitude of peak response ($\Delta_{Rmax\text{-}R0}$) and CPB duration would appear as predictors of DSB.

**Keywords:** cardiac surgery; milrinone; nebulizers; pharmacokinetics; pharmacodynamic.

## 11.2. Introduction

Cardiopulmonary bypass (CPB) is performed during cardiac surgery in order to maintain perfusion and oxygenation to all organs, besides the heart and lungs. Hemodynamic complications associated with difficult separation from bypass (DSB)[1] represent a leading cause of mortality in cardiac surgery.[2] Pulmonary hypertension (PH), as recently reviewed,[3] was identified as one of the most important hemodynamic predictor and risk factor for DSB.[4,5] Amongst other hemodynamic parameters used in cardiac surgery, the mean artery pressure (mAP) to mean pulmonary artery pressure (mPAP) ratio has proved to be the best predictor of perioperative complications.[6] In addition, the successful effect of inhaled therapy is expected to be associated with an increase in mAP/mPAP ratio and normalization of right ventricular function.[7-9] The mAP/mPAP ratio (R) remains unchanged following induction of general anesthesia[6] and correlates with the eccentricity index which reflects the interventricular septal deformation in response to PH.[10]

Intravenous milrinone is commonly used for the treatment of PH when DSB occurs at the end of cardiac surgery.[11-14] An important drawback of intravenous milrinone is its association with systemic hypotension.[15-17] Therefore, inhalation has been proposed as an alternative route of administration for milrinone.[18-20] Inhaled milrinone administered before CPB has also been proposed as having a protective effect during cardiac surgery[7,21-23] and a potential to facilitate separation from CPB in patients with PH.[24] However, the relationship between milrinone pharmacokinetics (PK) and pharmacodynamic (PD) following inhalation has never been characterized, especially using mAP/mPAP ratio as a PD marker.

This report on inhaled milrinone will present results obtained from a full-scale PK/PD study having three major objectives: characterization of inhaled milrinone PKs, exploration of the concentration-effect relationship and lastly, identification of potential predictors of DSB.

## 11.3. Materials and Methods

### 11.3.1. Patients

After approval by the institutional research ethic committee and with permission from Health Canada (ref: 108851), the study was registered in ClinicalTrials.gov (ref: NCT01725776). Written informed consent was obtained from 28 patients having preoperative PH and scheduled for elective cardiac surgery under CPB. Patients were considered having PH if either one of the following conditions was met before surgery: systolic pulmonary artery pressure (sPAP) >35 mmHg or mPAP >25 mmHg.[25] Patients with hemodynamic instability prior to surgery were excluded. Procedures were classified as coronary revascularization, valvular surgery or complex, defined as a combination of two or more different procedures. The EuroSCORE II was calculated for each patient.[26]

### 11.3.2. Surgical procedure

Patients were premedicated with 1-2 mg lorazepam orally 1 hour before surgery and received 0.1 mg $kg^{-1}$ morphine intramuscularly before entering the operating room where midazolam was given (0.01-0.05 mg $kg^{-1}$ intravenously) as needed for patient comfort. Usual monitoring was installed, including a 5-lead electrocardiogram, pulse oximeter, peripheral venous line, radial arterial line, 3-lumen catheter, and fast-response thermodilution pulmonary artery catheter. Anaesthesia was induced with 1 μg $kg^{-1}$ sufentanil and 0.04 mg $kg^{-1}$ midazolam, and muscle relaxation achieved with 0.1 mg $kg^{-1}$ pancuronium. After tracheal intubation, anaesthesia was maintained with 1 μg $kg^{-1}$ $h^{-1}$ sufentanil and 0.04 mg $kg^{-1}$ $h^{-1}$ midazolam. Intravenous fluids (0.9% normal saline) were administered according to estimated insensible losses of 7 cc $kg^{-1}$ $h^{-1}$ during surgery and titrated according to blood pressure and central venous pressure. A transesophageal echocardiography (TEE) omniplane probe was inserted after induction of general anaesthesia. Institution of CPB was performed using ascending aortic cannulation and bi-caval or double stage cannulation of the right atrium. Intermittent (4:1) blood cardioplegia was administered during CPB; induction and temperatures ranged from 15 to 29ºC. For coronary revascularizations, systemic temperature was allowed to drift to

34ºC, valvular surgeries and complex procedures to 32-34ºC. Weaning from CPB was undertaken after rewarming to a systemic temperature >36ºC.

## 11.3.3. Drug administration

After induction of anaesthesia, a TEE exam was conducted. Then, a 5 mg dose (50-80 $\mu g\ kg^{-1}$) of milrinone (Milrinone Lactate 1 mg $ml^{-1}$ (base); Pharmaceutical Partners of Canada Inc., Richmond Hill, ON, CAN) was administered by inhalation before initiation of CPB, using a mesh nebulizer (Aeroneb Professional Nebulizer System; Aerogen Ltd, Galway, Ireland). The nebulizer was attached to the inspiratory limb of the ventilator Y-connector near the endotracheal tube. Milrinone solution was placed into the nebulizer cup and inhalation was continued until aerosol production was deemed complete after gentle tapping of the device.

## 11.3.4. Pharmacokinetic study

### 11.3.4.1. PK sampling

Serial arterial blood draws (5 ml) were obtained before (blank; 0 min), during (2, 5, 10, 15 min) and immediately after termination of inhalation, as well as 3, 6, 9, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600 min thereafter. Two samples were also obtained 2 min after initiation and after weaning from CPB. Blood samples were kept on ice for a short period of time and centrifuged (3500 rpm; 15 min; 4°C). Plasma was immediately flash-frozen on dry ice and stored at -80°C until analysis. Milrinone plasma concentrations were determined by high performance liquid chromatography using tandem mass spectrometry detection (HPLC-MS/MS).[27] The lower limit of quantification (LLOQ) was 0.3125 ng $ml^{-1}$ with mean intra- and inter-assay precision bias (CV%) <12%.

### 11.3.4.2. Inhaled dose

Individual estimates of the inhaled dose ($D_{inh}$) after nebulization were determined using the approach previously described (please refer to manuscript Part I) and used for PK analysis. Milrinone treatment time, defined as the time required for complete nebulization, was closely monitored and nebulization rate was calculated.

#### 11.3.4.3. PK analysis

A two-compartment model with a zero-order input rate and elimination from the central compartment was fitted to individual inhaled milrinone plasma concentration–time profiles. Indeed, milrinone absorption process through pulmonary route is expected to be extremely rapid after inhalation (please refer to manuscript Part I). Relationship between milrinone systemic exposure and nebulization rate was also explored. The two-compartment model was selected after standard verification of its adequacy using the Akaike information criterion (AIC). Point estimates and PK parameters were optimized for individual data using a standard minimization method (Gauss–Newton, Levenberg and Hartley) and a weighting function of $1/\hat{y}$ (where $\hat{y}$ is the predicted concentration) was applied. Parameters including peak concentration (Cmax), peak time (Tmax), coefficients of bi-exponential equation describing disposition curve (A, B), fast distribution and elimination rate constants ($\alpha$, $\beta$), total body clearance and apparent volume of distribution expressed as a function of bioavailability (Cl/F, V/F) were determined using WinNonlin® Version 5.3 software (PK Model 10, Pharsight Corp., Mountain View, CA, USA).

### 11.3.5. Pharmacodynamic study

#### 11.3.5.1. PD endpoints

Hemodynamic parameters including mAP and mPAP were continuously monitored and data recorded at 1 and 15 min intervals during the pre- and post-CPB period, respectively. The mAP/mPAP ratio was later calculated and used as our PD marker. Surgical interventions, whenever possible, were avoided during the inhalation period. Important events and cutoff times used during data analysis are presented on a typical cardiac surgical procedure flowchart (Figure 1). For each patient, closed-chest baseline mAP/mPAP ratio ($R_0$) was determined from measures collected within 10 min immediately before inhalation (both mAP and mPAP had to be stable for at least 3 min). As baseline values are of paramount importance for PD analysis, $R_0$ values were rigorously determined by using the average value obtained from two independent experimenters. In order to eliminate any bias, this mean value was compared to the value measured at t=0 min and no significant difference was observed ($P$=0.441). This

approach for baseline characterization proved to be the most appropriate given that surgical interventions (TEE, legs raising, skin incision, or other surgical procedures) could not be avoided during the pre-inhalation period. Both open-chest peak mAP/mPAP ratio ($R_{max}$) and post-CPB mAP/mPAP ratio ($R_{post\text{-}CPB}$) were also considered as single point PD drivers. Another PD endpoint frequently used in our clinical setting that is the magnitude of peak response ($\Delta_{Rmax\text{-}R0}$) was also calculated. A one-way repeated measures analysis of variance (ANOVA) (SigmaPlot™ Version 11.2, Systat Software Inc., San Jose, CA, USA) was used to compare $R_0$, $R_{max}$ and $R_{post\text{-}CPB}$. Lastly, the relationship between these PD driver and DSB (clinical endpoint) was also explored.

#### 11.3.5.2. PK/PD analysis

Milrinone concentration-response relationship was analyzed by correlating patients' respective area under the plasma concentration-time curve (AUC) and area under the response-time curve (AUEC). Individual AUC and AUEC were calculated using the linear trapezoidal rule. For the calculation of AUEC, both positive and negative fluctuations from the predetermined baseline response ($R_0$; reference value) were taken into account during integration. Summation of all positive and negative partial AUEC yielded a net AUEC (NCA Model 220, Pharsight Corp., Mountain View, CA, USA). The AUEC-AUC relationship was investigated during the inhalation period (from 0 min until the end of inhalation). First, correlation was evaluated using all patients. The AUEC-intercept given by linear regression was considered to be the minimum threshold for response and considered as cut-off for determining responders. Then, correlation was re-evaluated in responders only. Finally, correlation between AUEC and $\Delta_{Rmax\text{-}R0}$ was verified and consistency of results confirmed.

#### 11.3.5.3. Clinical endpoint

The occurrence of DSB is considered as an important clinical endpoint in cardiac surgery. Two definitions were used to stratify the severity in weaning from CPB and were exclusive based on the type of support used from the end of CPB until the end of the surgery.[1] Easy separation from bypass was defined as either no support needed or

only one vasoactive (norepinephrine, phenylephrine, vasopressin) or inotropic (dobutamine, milrinone, epinephrine) agent being used. Difficult separation from bypass (DSB) was defined as the requirement for at least both vasoactive and inotropic agents or also defined as ≥1 failure of the first weaning attempt or the requirement for an intra-aortic balloon pump or a ventricular assist device to leave the operating room. As a secondary endpoint, we explored a plausible relationship between response to inhaled milrinone (selected single point PD drivers) and DSB. Because PH was identified as one of the most important hemodynamic predictor and risk factor for DSB,[4,5] a positive response to inhaled milrinone in attempt to control PH was considered a potential predictor of DSB. Simple and multiple logistic regressions were performed with stepwise selection (SigmaPlot™ Version 11.2, Systat Software Inc., San Jose, CA, USA) were used to identify potential predictors of DSB.

## 11.4. Results

A total of 28 patients were recruited. Demographic and perioperative data are shown in Table 1.

### 11.4.1. Pharmacokinetic study

#### 11.4.1.1. PK sampling

Individual milrinone plasma concentration-time profiles are presented in Figure 2. One patient was scheduled to receive elective cardiac surgery but did not undergo CPB (intraoperative decision) and was only considered for PK analysis during the inhalation period. Overall, Cmax values ranged between 41 and 189 ng $ml^{-1}$ and were observed at the end of inhalation. In all 28 patients plasma concentrations were quantifiable up to 10 hours after termination of inhalation.

#### 11.4.1.2. Inhaled dose

Mean estimated value for $D_{inh}$ in patients was 1.43 ± 0.45 mg (CV%=33%), representing 28.6% of the nominal dose. Mean residual and exhaled doses were 3.5 ± 1.2% (CV%=33%) and 26.4 ± 6.7% (CV%=25%), respectively. Milrinone average treatment time was 17 ± 6 min, ranging from 10 to 30 min. Mean nebulization rate was 0.086 ± 0.044 mg $min^{-1}$ (0.021-0.237 mg $min^{-1}$).

#### 11.4.1.3. PK analysis

Mean PK estimates obtained after fitting to data a two-compartment model (1/ŷ) with a zero-order input rate are presented in Table 2. Mean terminal elimination half-life was 154 ± 17 min. Milrinone systemic exposure was found to be inversely proportional to the nebulization rate ($r^2$=0.2524; *P*=0.006).

### 11.4.2. Pharmacodynamic study

#### 11.4.2.1. PD endpoints

One patient was not considered for PD analysis after unsuccessful Swan-Ganz installation. Figure 3 shows a typical plasma concentration-time profile and effect-time profile in one patient. For all patients, paired comparisons between $R_0$, $R_{max}$ and $R_{post\text{-}CPB}$ yielded a statistically significant difference (Figure 4A). Mean $\Delta_{Rmax\text{-}R0}$ was 0.58

representing a mean increase from baseline of 28% ($P$ <0.001). Otherwise, differences between $R_{post\text{-}CPB}$ and $R_0$ were less substantial with an average value of 0.20 (12%). Using a simple logistic regression, $\Delta_{Rmax\text{-}R0}$ was found to be directly related to the clinical endpoint DSB ($P$=0.009) (Figure 4B). When patients were categorized according to the occurrence of DSB, $\Delta_{Rmax\text{-}R0}$ mean increase from baseline was 0.37 (20%) in patients with DSB compared to 0.71 (32%) in patients without DSB ($P$=0.015).

#### 11.4.2.2. PK/PD analysis

During the inhalation period, the relationship between AUEC and AUC was best explained by a linear regression model ($r^2$=0.1513; $P$=0.045) (Figure 5A). The minimum threshold for therapeutic response in patients, i.e. the AUEC-intercept, was estimated as 1.387. Accordingly, 22 patients out of 27 were considered as responders. The exclusion of non-responders resulted in an improvement of this correlation ($r^2$=0.2292; $P$=0.024). Finally, the overall exposure to pharmacological response, AUEC, was also correlated with $\Delta_{Rmax\text{-}R0}$ ($r^2$=0.3568; $P$=0.001) (Figure 5B).

#### 11.4.2.3. Clinical endpoint

In an effort to develop a logistic model that would help predict the probability of DSB, several potential predictors were explored (EuroSCORE II, $R_0$, $R_{max}$, $\Delta_{Rmax\text{-}R0}$ and CPB duration). The predictive variables retained during forward analysis are presented in Table 3.

## 11.5. Discussion

To our knowledge, this is the first report attempting to characterize inhaled milrinone concentration-effect relationship. When the mAP/mPAP ratio (R) was used as PD marker, the magnitude of peak pharmacological response to inhaled milrinone before CPB ($\Delta_{Rmax-R0}$) and CPB duration were both identified as predictors of DSB. These results suggest that when inhaled milrinone is administered before CPB in cardiac surgery, the former clinical endpoint would represent a potential prognostic tool in predicting DSB.

For most routes of administration, the dose given to a patient is assumed to be completely delivered. This is often not the case for the pulmonary route, even after complete nebulization. This is even less the case for the inhaled dose which represents the fraction of the nominal dose that ultimately reaches the distal end of the endotracheal tube. In the context of cardiac surgery (*in vivo* setting), milrinone inhaled dose could not be directly measured and was estimated using an approach based on combined *in vivo* and *in vitro* data accounting for quantifiable and non-quantifiable losses within the respiratory apparatus, respectively. Since milrinone is almost completely excreted unchanged, urinary data (complete 24h-collection in a subset of 15 patients included herein) served as an external validation (please refer to manuscript Part I). According to this approach, a mean recovery of 95.3% of the nominal dose, which included the inhaled dose, exhaled dose and losses within the respiratory apparatus, was estimated. For these reasons, individual inhaled doses were estimated and used for PK analyses.

Given milrinone small molecular size (MW: 211.2), lipid solubility (log P: 1.17), as well as the large and well-perfused surface area provided by the lungs,[28] absorption process through the pulmonary route was expected to be extremely rapid (almost instantaneous).[29] Indeed, many small molecules have pulmonary bioavailability approaching 100%,[30-32] which can be attributed to their rapid absorption from the lungs and low concentrations of drug-metabolizing enzymes in the lungs compared to the oral route.[33-35] Inhaled prochlorperazine is an example of a rapidly absorbed drug that resulted in superimposed plasma concentration-time profiles after nebulization or intravenous administration in both anesthetized dogs[36] and humans.[37] Accordingly, a

two-compartment model with a zero-order input was deemed adequate. In agreement with the literature for this type of device,[38] milrinone treatment time varied greatly amongst our patients after mesh nebulization (10-30 min). Moreover, milrinone systemic exposure was significantly reduced at higher nebulization rate, reinforcing the necessity of a close monitoring to document nebulization duration.

Both non-compartmental analysis (please refer to manuscript Part I) and compartmental analyses yielded similar PK parameters and agreed with those reported after IV administration in congestive heart failure patients[39,40] and patients undergoing cardiac surgery,[41-43] suggesting a rapid and complete absorption of milrinone through the lungs. In contrast to pediatric patients undergoing cardiac surgery using CPB,[44] the presence of CPB in adult patients does not seem to affect milrinone PK significantly. This observation was also reported by others[41,45] after comparing PK parameters with those obtained in congestive heart failure patients[41] or plasma concentration-time profiles obtained when milrinone was administered before vs after CPB in cardiac patients.[45]

The mAP/mPAP ratio was chosen as our PD marker mostly on the basis of sounded evidence for its prognostic value as the best predictor of perioperative complications in cardiac surgery.[6] Previous studies[7,8] and case report[9] described how increases in the ratio following administration of inhaled agents in patients are associated with improvement of the right ventricular function. Moreover, the mAP/mPAP ratio is correlated with the eccentricity index which is the intraventricular deformation resulting from PH.[10] Hence, relative instead of absolute values of mPAP are commonly used for estimation of PH in congenital cardiology.[46,47] A normal value for mAP/mPAP ratio is generally expected to be greater than 4; thus, lower values are good indicators of the severity of PH. In patients with preoperative PH, induction of general anesthesia was reported to result in a significant reduction in both mAP and mPAP while mAP/mPAP ratio remained stable.[6] Thus, in patients under general anesthesia and in absence of surgical stress, the mAP/mPAP ratio should change proportionally to any alteration of PH.

Time-specific single point measures of the intensity of effect represented by $R_0$, $R_{max}$, $R_{post\text{-}CPB}$, as well as $\Delta_{Rmax\text{-}R0}$ have already been used as hemodynamic endpoints in cardiac surgery for patients with PH.[7] In our patients, the mean increase in $R_{post\text{-}CPB}$ was

not significant at the end of CPB when compared to $R_0$ ($P$=0.358). At this time-point, it is difficult to distinguish the effect attributable to milrinone residual pharmacological effect from that induced by hemodynamic changes associated with CPB weaning. In our opinion, the magnitude of effect obtained at the end of inhalation, expressed as $\Delta_{Rmax\text{-}R0}$, is more relevant because mostly attributable to the PD marker. It is worth pointing out that both $R_{max}$ and $R_{post\text{-}CPB}$ were opened-chest measures while $R_0$ was determined at closed-chest. For instance, mean value for $R_{post\text{-}CPB}$ was 2.70 when measured after chest closure compared to 2.27 before chest closure. Thus, estimation of $\Delta_{Rmax\text{-}R0}$ mean value is conservative and would have been higher if $R_{max}$ could have been taken under closed-chest conditions.

After examination of raw PD data, stress produced by surgical interventions carried immediately before and/or during inhalation caused unpredictable variations in mAP/mPAP ratios, compromising accurate PK/PD characterization. For a given patient, no relationship was observed between raw mAP/mPAP ratios and milrinone plasma concentrations nor between $R_{max}$ or $\Delta_{Rmax\text{-}R0}$ values and peak concentration. It was felt that, rather than looking at separate measurements over time, a more accurate estimate of the overall effect would be obtained by integrating effect over time.[48] Such an approach would be more appropriate for assessing the net pharmacologic response to a given dose in presence of PD fluctuations.[49] For this reason, AUEC was used to evaluate the extent of PD response for each patient.

A linear relationship between milrinone systemic exposure (AUC) and the corresponding pharmacologic effect exposure (AUEC) during inhalation would represent the first step towards establishment of a potential proof of concept. Higher occurrence of surgical artifacts and higher variability in time elapsed before starting CPB (15-125 min) as well as CPB durations (39-325 min) compared to inhalation durations (10-30 min) may explain why this linear relationship was not maintained after inhalation. Keeping in mind that the overarching goal is to obtain a readily accessible PD driver that would adequately reflect milrinone overall effect during the inhalation period, the significant correlation observed between AUEC (exposure) and $\Delta_{Rmax\text{-}R0}$ (single point) suggests that the overall net effect is in agreement with the magnitude of

response obtained at peak effect (end of inhalation). Accordingly, non-responders showed both low AUEC and low $\Delta_{Rmax-R0}$ values. Other studies on inhaled milrinone administered prior to CPB in cardiac surgery have also observed 18-26% of non-responsers amongst their population of pulmonary hypertensive patients.[7,22,24] This could be explained by the fact that chronic hypoxia and vascular remodeling is assumed to result in secondary and in some cases fixed pre-capillary PH, which is an independent predictor of mortality.[50]

Finally, as the occurrence of DSB represents the major clinical endpoint in cardiac surgery for procedures requiring CPB, several potential predictors were explored and considered in a multiple logistic regression model for DSB. AUEC was not retained for establishing a link with the clinical response mostly because these values are not readily obtainable during surgery as they need to be computed. Therefore, in view of future clinical applications, single point PD drivers available prior to CPB weaning ($R_0$, $R_{max}$ and $\Delta_{Rmax-R0}$) were explored to identify potential predictors of outcome readily accessible in a clinical setting. A logistic regression model was used to predict the probability of DSB following prophylactic administration of inhaled milrinone in patients undergoing cardiac surgery. The objective was to identify variables with potential predictive value for our clinical outcome (DSB). Therefore, prognostic variables were defined as variables that could be obtained before CPB. Variable selection was also based on clinical relevance that is prior knowledge of the pathophysiology related to CPB and factors susceptible to impact on its outcome. Accordingly, tested variables were related to the patient's health status (severity of HP and euroSCORE), to surgery (CPB duration) or to patient's response to inhaled milrinone ($R_0$, $R_{max}$, $\Delta_{Rmax-R0}$, AUEC). Amongst variables tested during univariate analysis, only those that proved to be statistically significant were retained for multivariate analysis. Only the magnitude of peak response ($\Delta_{Rmax-R0}$) and CPB duration remained in the final model. In a previous study, CPB duration was also identified as a strong risk factor of DSB.[24]

The major limitation of this study was the impossibility of modeling each patient's whole set of concentration-effect data because PD data were often contaminated by

surgical interventions. Moreover, inclusion criteria allowed a wide range of PH (sPAP 36-90 mmHg) and study population was not quite homogeneous for a phase IIa (euroSCORE 1.2-46.4). Milrinone dose may also have been suboptimal (taking into account the inhaled dose measured) and may have to be increased in further dose-ranging studies. In absence of rich data PK/PD analysis, our sample size may not have been sufficient and a larger scale study would be required. Despite our limited sample size, the magnitude of peak pharmacological response ($\Delta_{Rmax-R0}$) and CPB duration were both found to be predictors of DSB.

In summary, this is the first study reporting PK and PD data obtained after inhalation of milrinone in cardiac surgical patients. Comparison of respective milrinone AUC and AUEC before CPB provided preliminary evidence of a proof of concept for the use of the mAP/mPAP ratio as a promising PD driver and warrants future studies. Magnitude of peak pharmacological response ($\Delta_{Rmax-R0}$) and CPB duration were both found to be predictors of DSB.

## 11.6. References


1. Denault AY, Tardif JC, Mazer CD, Lambert J: Difficult and complex separation from cardiopulmonary bypass in high-risk cardiac surgical patients: a multicenter study. J Cardiothorac Vasc Anesth 2012; 26: 608-16

2. Reich DL, Bodian CA, Krol M, Kuroda M, Osinski T, Thys DM: Intraoperative hemodynamic predictors of mortality, stroke, and myocardial infarction after coronary artery bypass surgery. Anesth Analg 1999; 89: 814-22

3. Nguyen AQ, Deschamps A, Denault AY, Varin F, Perrault LP: A Pathophysiological Approach to Understanding Pulmonary Hypertension in Cardiac Surgery, Perioperative Considerations in Cardiac Surgery. Edited by Cuneyt Narin. Rijeka, INTECH Open Access Publisher, 2012, pp 277-306

4. Tuman KJ, McCarthy RJ, March RJ, Najafi H, Ivankovich AD: Morbidity and duration of ICU stay after cardiac surgery. A model for preoperative risk assessment. Chest 1992; 102: 36-44

5. Malouf JF, Enriquez-Sarano M, Pellikka PA, Oh JK, Bailey KR, Chandrasekaran K, Mullany CJ, Tajik AJ: Severe pulmonary hypertension in patients with severe aortic valve stenosis: clinical profile and prognostic implications. J Am Coll Cardiol 2002; 40: 789-95

6. Robitaille A, Denault AY, Couture P, Belisle S, Fortier A, Guertin MC, Carrier M, Martineau R: Importance of relative pulmonary hypertension in cardiac surgery: the mean systemic-to-pulmonary artery pressure ratio. J Cardiothorac Vasc Anesth 2006; 20: 331-9

7. Denault A, Haddad F, Lamarche Y, Nguyen A, Varin F: Pilot randomized controlled trial of inhaled milrinone in high-risk cardiac surgical patients. Surgery Curr Res 2014; 4: 192

8. Laflamme M, Perrault LP, Carrier M, Elmi-Sarabi M, Fortier A, Denault AY: Preliminary experience with combined inhaled milrinone and prostacyclin in cardiac surgical patients with pulmonary hypertension. J Cardiothorac Vasc Anesth 2015; 29: 38-45

9. St-Pierre P, Deschamps A, Cartier R, Basmadjian AJ, Denault AY: Inhaled Milrinone and Epoprostenol in a Patient With Severe Pulmonary Hypertension, Right Ventricular Failure, and Reduced Baseline Brain Saturation Value From a Left Atrial Myxoma. J Cardiothorac Vasc Anesth 2013

10. Haddad F, Guihaire J, Skhiri M, Denault AY, Mercier O, Al-Halabi S, Vrtovec B, Fadel E, Zamanian RT, Schnittger I: Septal curvature is marker of hemodynamic, anatomical, and electromechanical ventricular interdependence in patients with pulmonary arterial hypertension. Echocardiography 2014; 31: 699-707

11. Doolan LA, Jones EF, Kalman J, Buxton BF, Tonkin AM: A placebo-controlled trial verifying the efficacy of milrinone in weaning high-risk patients from cardiopulmonary bypass. J Cardiothorac Vasc Anesth 1997; 11: 37-41

12. Solina A, Papp D, Ginsberg S, Krause T, Grubb W, Scholz P, Pena LL, Cody R: A comparison of inhaled nitric oxide and milrinone for the treatment of pulmonary hypertension in adult cardiac surgery patients. J Cardiothorac Vasc Anesth 2000; 14: 12-7

13. Feneck RO, Sherry KM, Withington PS, Oduro-Dominah A: Comparison of the hemodynamic effects of milrinone with dobutamine in patients after cardiac surgery. J Cardiothorac Vasc Anesth 2001; 15: 306-15

14. Solina AR, Ginsberg SH, Papp D, Grubb WR, Scholz PM, Pantin EJ, Cody RP, Krause TJ: Dose response to nitric oxide in adult cardiac surgery patients. J Clin Anesth 2001; 13: 281-6

15. Jaski BE, Fifer MA, Wright RF, Braunwald E, Colucci WS: Positive inotropic and vasodilator actions of milrinone in patients with severe congestive heart failure. Dose-response relationships and comparison to nitroprusside. J Clin Invest 1985; 75: 643-9

16. Cuffe MS, Califf RM, Adams KF, Jr., Benza R, Bourge R, Colucci WS, Massie BM, O'Connor CM, Pina I, Quigg R, Silver MA, Gheorghiade M: Short-term intravenous milrinone for acute exacerbation of chronic heart failure: a randomized controlled trial. Jama 2002; 287: 1541-7

17. Couture P, Denault AY, Pellerin M, Tardif JC: Milrinone enhances systolic, but not diastolic function during coronary artery bypass grafting surgery. Can J Anaesth 2007; 54: 509-22

18. Haraldsson A, Kieler-Jensen N, Ricksten SE: The additive pulmonary vasodilatory effects of inhaled prostacyclin and inhaled milrinone in postcardiac surgical patients with pulmonary hypertension. Anesth Analg 2001; 93: 1439-45

19. Sablotzki A, Starzmann W, Scheubel R, Grond S, Czeslick EG: Selective pulmonary vasodilation with inhaled aerosolized milrinone in heart transplant candidates. Can J Anaesth 2005; 52: 1076-82

20. Wang H, Gong M, Zhou B, Dai A: Comparison of inhaled and intravenous milrinone in patients with pulmonary hypertension undergoing mitral valve surgery. Adv Ther 2009; 26: 462-8

21. Lamarche Y, Malo O, Thorin E, Denault A, Carrier M, Roy J, Perrault LP: Inhaled but not intravenous milrinone prevents pulmonary endothelial dysfunction after cardiopulmonary bypass. J Thorac Cardiovasc Surg 2005; 130: 83-92

22. Hegazy N, Elhenawy A: Comparison of Hemodynamic Effects of Inhaled Milrinone and Inhaled Prostacyclin after Adult Cardiac Surgery. J Appl Sci Res 2010; 6: 38-44

23. Gong M, Lin XZ, Lu GT, Zheng LJ: Preoperative inhalation of milrinone attenuates inflammation in patients undergoing cardiac surgery with cardiopulmonary bypass. Med Princ Pract 2012; 21: 30-5

24. Lamarche Y, Perrault LP, Maltais S, Tetreault K, Lambert J, Denault AY: Preliminary experience with inhaled milrinone in cardiac surgery. Eur J Cardiothorac Surg 2007; 31: 1081-7

25. Moraes D, Loscalzo J: Pulmonary hypertension: newer concepts in diagnosis and management. Clin Cardiol 1997; 20: 676-82

26. Nashef SA, Roques F, Sharples LD, Nilsson J, Smith C, Goldstone AR, Lockowandt U: EuroSCORE II. Eur J Cardiothorac Surg 2012; 41: 734-44; discussion 744-5

27. Gavra P, Nguyen AQ, Theoret Y, Litalien C, Denault AY, Varin F: A specific and sensitive HPLC-MS/MS micromethod for milrinone plasma levels determination after inhalation in cardiac patients. Ther Drug Monit 2014; 36: 663-8

28. Weibel ER: Morphometry of the human lung. New York, Academic Press, 1963

29. Patton JS, Byron PR: Inhaling medicines: delivering drugs to the body through the lungs. Nat Rev Drug Discov 2007; 6: 67-74

30. Patton JS: Mechanisms of macromolecule absorption by the lungs. Advanced Drug Delivery Reviews 1996; 19: 3-36

31. Brown RA, Jr., Schanker LS: Absorption of aerosolized drugs from the rat lung. Drug Metab Dispos 1983; 11: 355-60

32. Schanker LS, Mitchell EW, Brown RA, Jr.: Species comparison of drug absorption from the lung after aerosol inhalation or intratracheal injection. Drug Metab Dispos 1986; 14: 79-88

33. Keith IM, Olson EB, Jr., Wilson NM, Jefcoate CR: Immunological identification and effects of 3-methylcholanthrene and phenobarbital on rat pulmonary cytochrome P-450. Cancer Res 1987; 47: 1878-82

34. Ji CM, Cardoso WV, Gebremichael A, Philpot RM, Buckpitt AR, Plopper CG, Pinkerton KE: Pulmonary cytochrome P-450 monooxygenase system and Clara cell differentiation in rats. Am J Physiol 1995; 269: L394-402

35. Tronde A, Norden B, Marchner H, Wendel AK, Lennernas H, Bengtsson UH: Pulmonary absorption rate and bioavailability of drugs in vivo in rats: structure-absorption relationships and physicochemical profiling of inhaled drugs. J Pharm Sci 2003; 92: 1216-33

36. Avram MJ, Henthorn TK, Spyker DA, Krejcie TC, Lloyd PM, Cassella JV, Rabinowitz JD: Recirculatory pharmacokinetic model of the uptake, distribution, and bioavailability of prochlorperazine administered as a thermally generated aerosol in a single breath to dogs. Drug Metab Dispos 2007; 35: 262-7

37. Rabinowitz JD, Lloyd PM, Munzar P, Myers DJ, Cross S, Damani R, Quintana R, Spyker DA, Soni P, Cassella JV: Ultra-fast absorption of amorphous pure drug aerosols via deep lung inhalation. J Pharm Sci 2006; 95: 2438-51

38. Dhand R: Nebulizers that use a vibrating mesh or plate with multiple apertures to generate aerosol. Respir Care 2002; 47: 1406-16; discussion 1416-8

39. Stroshane RM, Koss RF, Biddlecome CE, Luczkowec C, Edelson J: Oral and intravenous pharmacokinetics of milrinone in human volunteers. J Pharm Sci 1984; 73: 1438-41

40. Edelson J, Stroshane R, Benziger DP, Cody R, Benotti J, Hood WB, Jr., Chatterjee K, Luczkowec C, Krebs C, Schwartz R: Pharmacokinetics of the bipyridines amrinone and milrinone. Circulation 1986; 73: III145-52

41. Das PA, Skoyles JR, Sherry KM, Peacock JE, Fox PA, Woolfrey SG: Disposition of milrinone in patients after cardiac surgery. Br J Anaesth 1994; 72: 426-9

42. De Hert SG, Moens MM, Jorens PG, Delrue GL, DePaep RJ, Vermeyen KM: Comparison of two different loading doses of milrinone for weaning from cardiopulmonary bypass. J Cardiothorac Vasc Anesth 1995; 9: 264-71

43. Butterworth JF, Hines RL, Royster RL, James RL: A pharmacokinetic and pharmacodynamic evaluation of milrinone in adults undergoing cardiac surgery. Anesth Analg 1995; 81: 783-92

44. Bailey JM, Miller BE, Lu W, Tosone SR, Kanter KR, Tam VK: The pharmacokinetics of milrinone in pediatric patients after cardiac surgery. Anesthesiology 1999; 90: 1012-8

45. Bailey JM, Levy JH, Kikura M, Szlam F, Hug CC, Jr.: Pharmacokinetics of intravenous milrinone in patients undergoing cardiac surgery. Anesthesiology 1994; 81: 616-22

46. Therrien J, Dore A, Gersony W, Iserin L, Liberthson R, Meijboom F, Colman JM, Oechslin E, Taylor D, Perloff J, Somerville J, Webb GD: CCS Consensus Conference 2001 update: recommendations for the management of adults with congenital heart disease. Part I. Can.J.Cardiol. 2001; 17: 940-959

47. Therrien J, Gatzoulis M, Graham T, Bink-Boelkens M, Connelly M, Niwa K, Mulder B, Pyeritz R, Perloff J, Somerville J, Webb GD: Canadian Cardiovascular Society Consensus Conference 2001 update: Recommendations for the Management of Adults with Congenital Heart Disease--Part II. Can.J.Cardiol. 2001; 17: 1029-1050

48. Kwon Y: Handbook of essential pharmacokinetics, pharmacodynamics and drug metabolism for industrial scientists, Springer Science & Business Media, 2001

49. Krzyzanski W, Jusko WJ: Integrated functions for four basic models of indirect pharmacodynamic response. J Pharm Sci 1998; 87: 67-72

50. Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, Gomez Sanchez MA, Krishna Kumar R, Landzberg M, Machado RF, Olschewski H, Robbins IM, Souza R: Updated clinical classification of pulmonary hypertension. J Am Coll Cardiol 2013; 62: D34-41

## 11.7. Tables

**Table 1.** Patient demographic and perioperative data

| | | | |
|---|---|---|---|
| Gender (f : m) | 12 | : | 16 |
| Age (yr) | 67 | ± | 10 |
| Weight (kg) | 72 | ± | 10 |
| Height (cm) | 165 | ± | 7 |
| sPAP (mmHg) | 65 | ± | 17 |
| EuroSCORE II | 8.0 | ± | 8.7 |
| Type of surgical procedure | | | |
| CABG | 1 | | |
| Single valve | 12 | | |
| Complex | 13 | | |
| Other | 2 | | |
| Milrinone nebulization time (min) | 17 | ± | 6 |
| CPB duration (min) | 116 | ± | 72 |
| DSB (y : n) | 10 | : | 17 |

Values expressed as mean ± SD (n=28).
CABG, coronary artery bypass graft; CPB, cardiopulmonary bybass; DSB, difficult separation from bypass; sPAP, systolic pulmonary arterial pressure.

**Table 2.** Milrinone PK parameters after fitting a two-compartment model (1/ŷ) with zero-order input to individual data

| $V_c/F$ (L $kg^{-1}$) | $V_{ss}/F$ (L $kg^{-1}$) | Cl/F (L $h^{-1}$ $kg^{-1}$) | A | B | α ($min^{-1}$) | β ($min^{-1}$) |
|---|---|---|---|---|---|---|
| 0.12 ± 0.06 | 0.39 ± 0.25 | 0.11 ± 0.05 | 162 ± 89 | 42 ± 20 | 0.0944 ± 0.0984 | 0.0042 ± 0.0015 |

Values expressed as mean ± SD (n=22).

Vc, apparent volume of distribution of central compartment; F, bioavailability; Vss, apparent volume of distribution at steady-state; Cl, total body clearance; A, coefficient of biexponential equation describing distribution curve; B, coefficient of biexponential equation describing elimination curve; α, distribution rate constant; β, elimination rate constant.

**Table 3.** Determination of explanatory variables in a logistic model for DSB

| Model variables | n | "-2LL" | LRT *P*-value | Δ(-2LL) |
|---|---|---|---|---|
| **STEP 1** | | | | |
| DSB + … | | | | |
| effect of EuroSCORE II | 27 | 31.223 | 0.037 | |
| effect of $R_0$ | 26 | 34.129 | 0.472 | |
| effect of $R_{max}$ | 26 | 31.930 | 0.099 | |
| effect of $\Delta_{Rmax-R0}$ | 26 | 27.745 | 0.009 | |
| effect of CPB duration | 27 | 22.443 | <0.001 | |
| **STEP 2** | | | | |
| DSB + effect of CPB duration + … | | | | |
| effect of $\Delta_{Rmax-R0}$ | 26 | 17.574 | <0.001 | -4.869 * |

DSB, difficult separation from bypass; "-2LL", -2Log(Likelihood); LRT, Likelihood Ratio Test; Δ(-2LL), decrease in objective function (-2LL); $R_0$, baseline mAP/mPAP ratio; $R_{max}$, peak mAP/mPAP ratio; $\Delta_{Rmax-R0}$, magnitude of peak response; CPB, cardiopulmonary bypass.

*$P$ <0.05, Δ(-2LL) >3.84

## 11.8. Figures

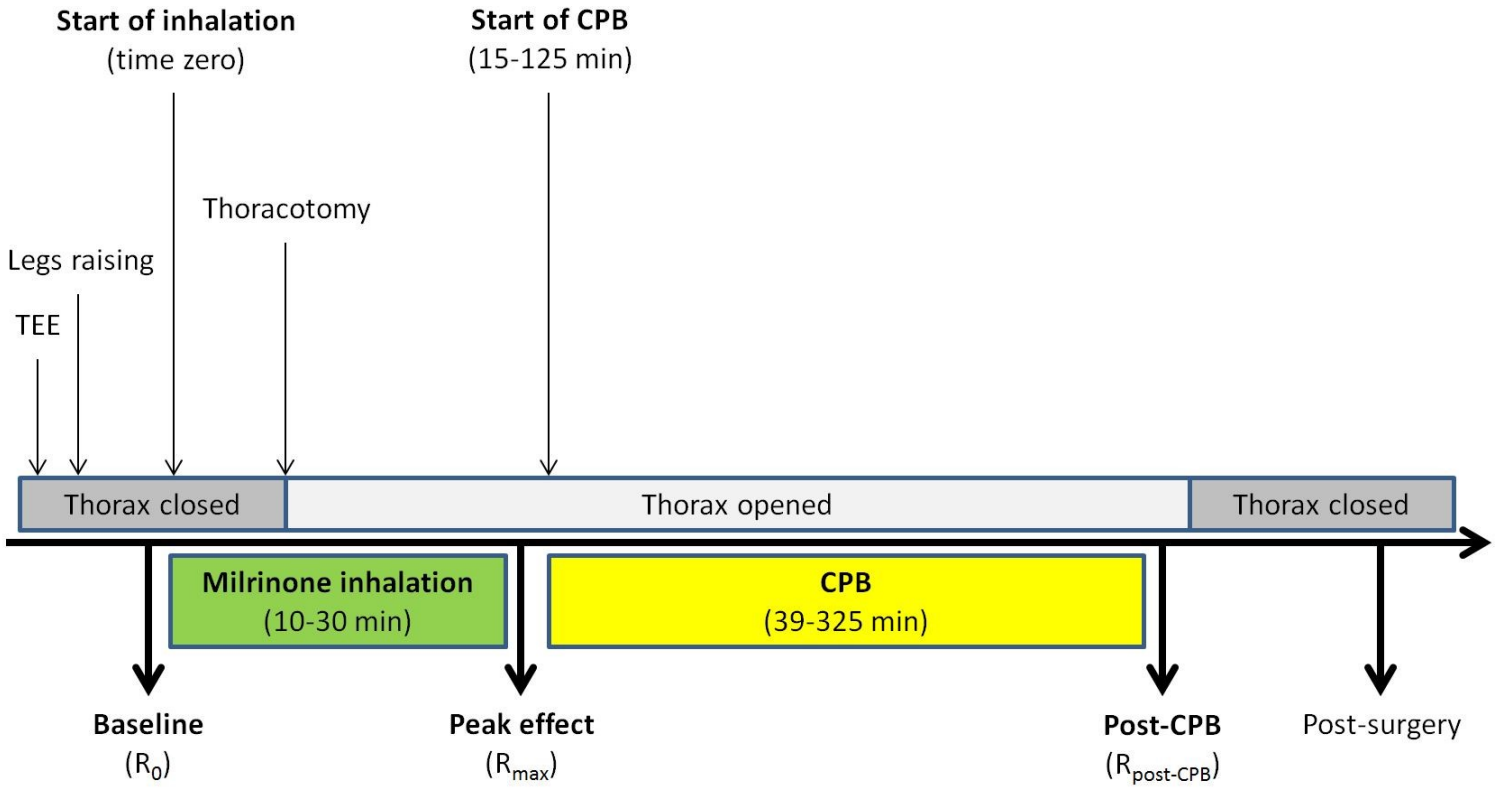


**Figure 1.** Important events and cut off times used for data analysis on a typical cardiac surgical procedure time flow chart. TEE, transesophageal echocardiographic exam; Emax, maximum effect; CPB, cardiopulmonary bypass; $R_0$, baseline mAP/mPAP ratio; $R_{max}$, peak mAP/mPAP ratio; $R_{post\text{-}CPB}$, post-CPB mAP/mPAP ratio.

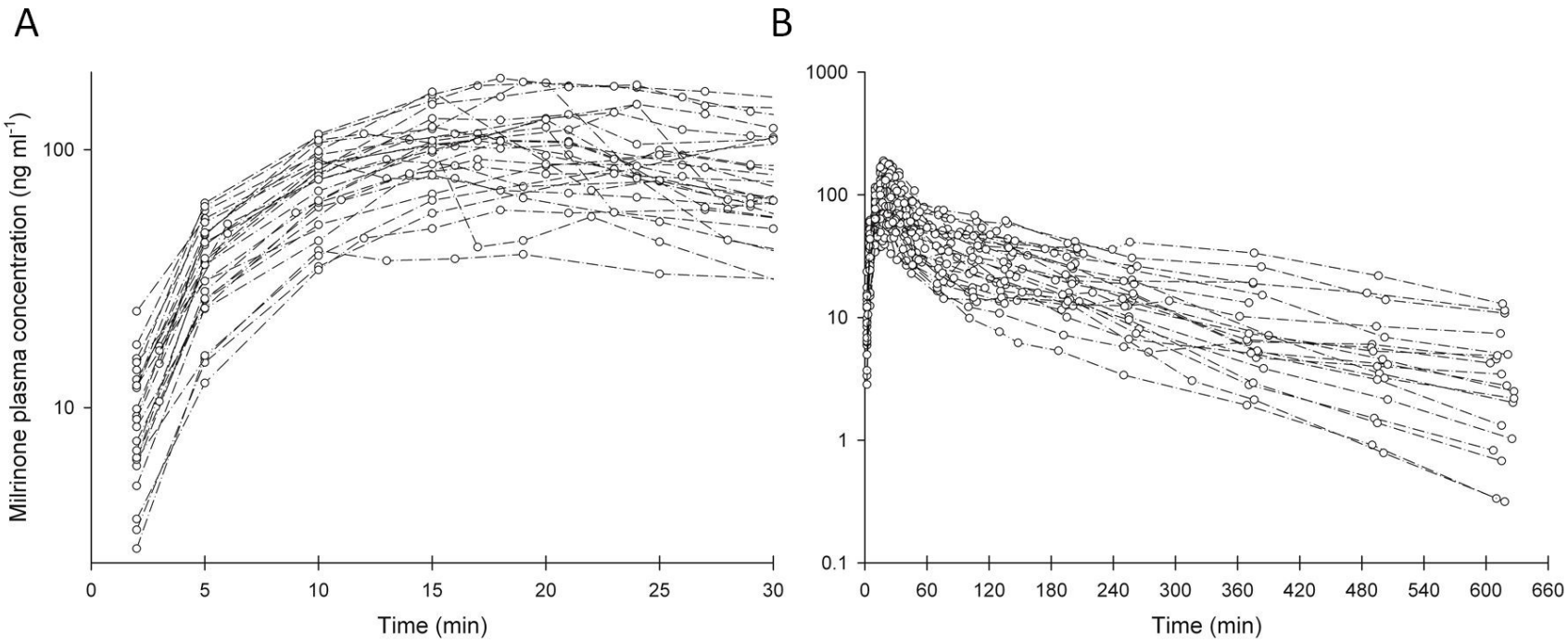


**Figure 2.** Individual milrinone plasma concentration-time profiles during inhalation (10-30 min) (**A**) and overall until 600 min after termination of inhalation (**B**) (n=28).

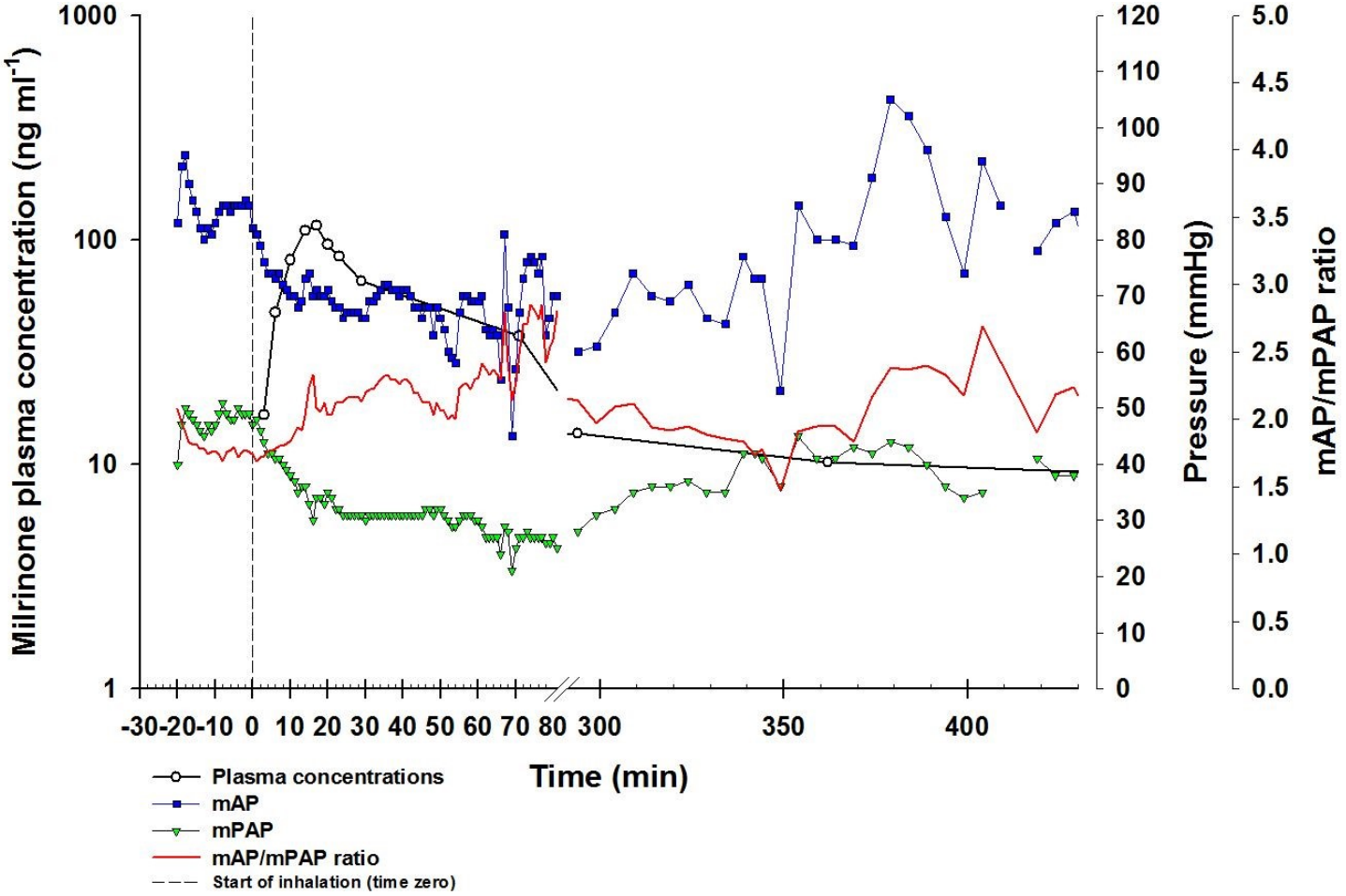


**Figure 3.** Typical plasma concentration-time profile and effect-time profile for one patient. mAP, mean arterial pressure; mPAP, mean pulmonary arterial pressure.

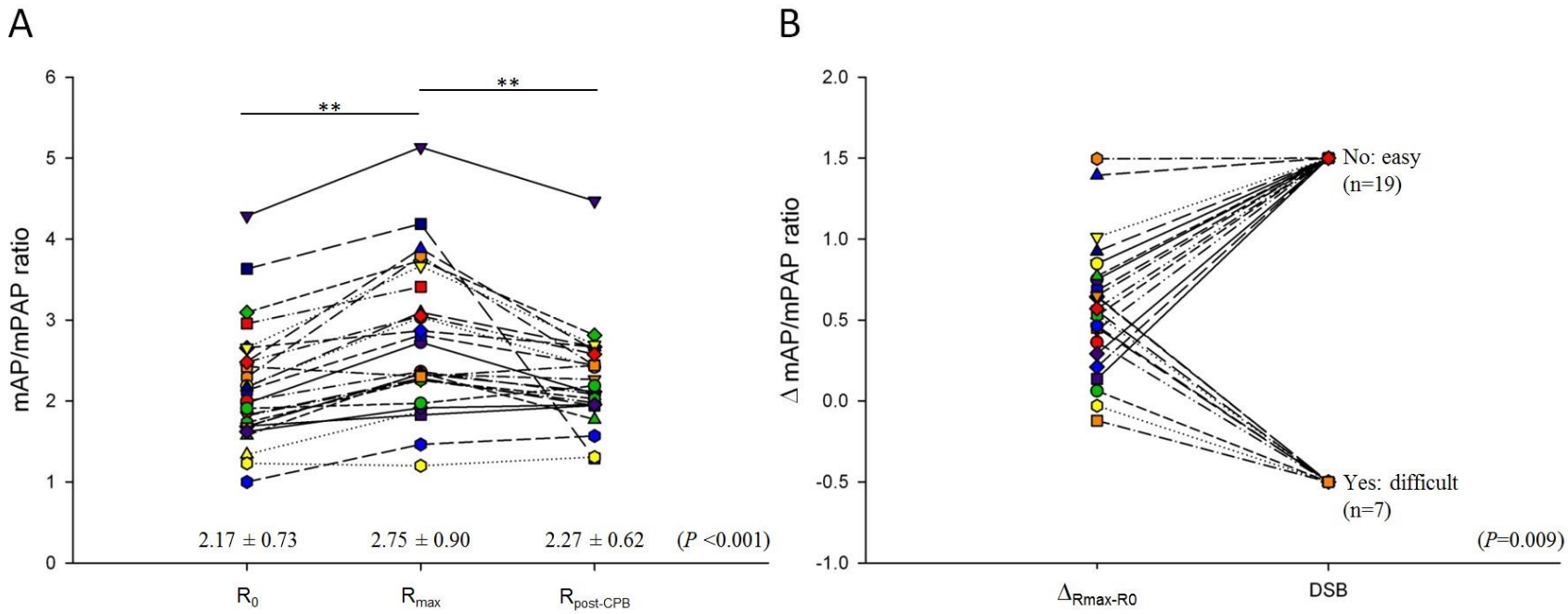


**Figure 4.** Association between $R_0$ (n=27), $R_{max}$ (n=27) and $R_{post\text{-}CPB}$ (n=25) using one-way repeated measures analysis of variance (ANOVA) (**A**) and association between $\Delta_{Rmax\text{-}R0}$ and DSB (clinical endpoint) using simple logistic regression (**B**). mAP, mean arterial pressure; mPAP, mean pulmonary arterial pressure; $R_0$, baseline mAP/mPAP ratio; $R_{max}$, peak mAP/mPAP ratio; $R_{post\text{-}CPB}$, post-CPB mAP/mPAP ratio; $\Delta_{Rmax\text{-}R0}$, magnitude of peak response; DSB, difficult separation from bypass. Mean ± SD **$P$ <0.001

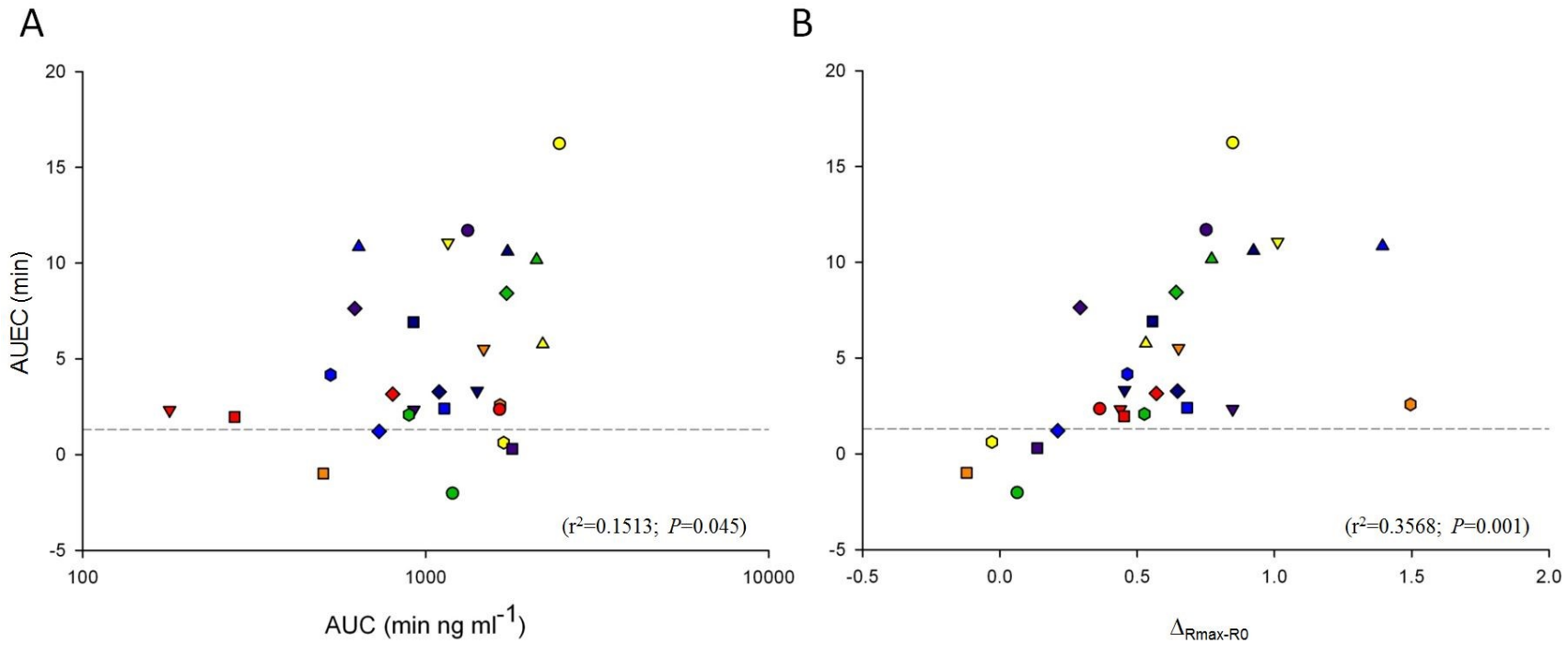


**Figure 5.** Relationship between AUEC and AUC (**A**) and relationship between AUEC and $\Delta_{Rmax\text{-}R0}$ (**B**) for the inhalation period (10-30 min) using linear regression models (n=27). AUEC, area under the effect-time curve; AUC, area under the plasma concentration-time curve; $\Delta_{Rmax\text{-}R0}$, magnitude of peak response.

# SECTION III: CONCLUSION

# CHAPITRE 12. Discussion générale

Les travaux de cette thèse avaient pour objectif principal d'étudier la relation pharmacocinétique/pharmacodynamique (PK/PD) de la milrinone inhalée chez des patients souffrant d'hypertension pulmonaire et qui doivent subir une chirurgie cardiaque sous circulation extracorporelle (CEC). L'approche utilisée reposait sur l'utilisation du ratio de la pression artérielle moyenne sur la pression artérielle pulmonaire moyenne (PAm/PAPm) comme biomarqueur PD dans l'établissement d'une relation exposition-réponse qui relie les aires sous la courbe des concentrations plasmatiques en fonction du temps (ASC) avec les aires sous la courbe de l'effet pharmacologique en fonction du temps (ASCE).

Le volet pharmaceutique de ce projet de doctorat avait comme premier objectif d'optimiser une méthode analytique (spécifique, sensible et reproductible) permettant de quantifier la milrinone plasmatique suite à son administration par voie inhalée. Le deuxième objectif de ce volet consistait à estimer la dose inhalée et déterminer l'exposition systémique en explorant la PK de la milrinone suite à l'inhalation de la solution intraveineuse au moyen d'un nébuliseur chez des patients subissant une chirurgie cardiaque sous CEC.

Le volet clinique se voulait tout d'abord de caractériser la relation PK/PD de la milrinone inhalée à l'aide du biomarqueur PD. L'objectif à long terme ayant une application clinique importante était d'explorer si la réponse à la milrinone pouvait potentiellement prédire une sortie de CEC difficile chez les patients lors du rétablissement de la circulation sanguine à la fin de la chirurgie cardiaque (efficacité clinique).

## 12.1. Choix de la méthode analytique

Le choix de la méthode analytique utilisée pour la quantification d'un médicament dépend de plusieurs facteurs et varie selon les besoins de chaque étude. Outre les principaux critères de spécificité et de sensibilité nécessaires à la couverture complète du

profil pharmacocinétique d'un médicament, l'accessibilité à l'équipement requis exerce également une grande influence sur le choix de la méthode.

Lorsque le projet de recherche a débuté, la plupart des méthodes publiées pour le dosage de la milrinone dans le plasma et dans l'urine utilisaient un appareil de type HPLC-UV. Puisque cette technologie était disponible dans notre laboratoire et que nous étions limités par un manque de sensibilité, notre stratégie a consisté à optimiser une des méthodes existantes afin de lui permettre d'atteindre une limite de quantification inférieure à 5 ng/ml (chapitre 9, manuscrit n˚2). Suite à quelques analyses préliminaires effectuées sur les échantillons plasmatiques obtenus chez nos premiers patients, nous avions jugé qu'une limite de quantification de 1.25 ng/ml serait suffisante pour caractériser la PK de la milrinone inhalée. Une fois optimisée et validée, cette nouvelle méthode nous a permis de doser la milrinone plasmatique chez les patients inclus dans notre première étude clinique (chapitre 10, manuscrit n˚3).

Au cours de la deuxième étude clinique (chapitre 11, manuscrit n˚4), des interférences chromatographiques ont commencé à être observées au temps de rétention de la milrinone, nuisant ainsi à la détermination quantitative adéquate du médicament. Ceci peut s'expliquer, en partie, par une différence de l'état de santé des patients dans la population de la seconde étude. En effet, la plupart des patients étaient plus malades et recevaient plus souvent d'autres traitements concomitants avant, pendant et après la CEC. De plus, notre problème de manque de sensibilité persistait puisque les derniers échantillons collectés 8-10 h post-milrinone chez quelques patients se sont trouvés sous la limite de quantification. Enfin, nous avons également noté l'apparition d'une variation intersujet pour notre standard interne (amrinone), ce qui induisait un biais systématique lorsque les échantillons cliniques étaient comparés à ceux des standards de la courbe de calibration. Pour toutes les raisons mentionnées, nous avons décidé de développer une nouvelle méthode analytique au laboratoire du Dr Yves Théoret du département de biochimie du CHU Sainte-Justine (annexe II, manuscrit n˚5 coauteur).[165] Brièvement, cette méthode utilise une technologie de détection plus avancée (MS/MS) nous permettant d'acquérir une spécificité significativement supérieure ainsi qu'une meilleure sensibilité (0.3125 ng/ml) avec un temps d'analyse 5 fois plus rapide (2.6 min) et un

volume plasmatique 20 fois moindre (50 µl). Afin de régler notre problème de stabilité du standard interne, l'amrinone a été remplacée par l'olprinone, un analogue plus stable dans nos conditions analytiques.

## 12.2. Estimation de la dose inhalée

Les nébuliseurs sont des dispositifs d'inhalation de plus en plus utilisés pour l'administration des médicaments chez les patients soumis à une ventilation mécanique. Toutefois, le système de ventilation artificielle exerce un impact particulier sur la genèse et le transport des goutelettes aérosolisées en introduisant des nouveaux facteurs qui peuvent altérer le rendement du système de nébulisation. Par conséquent, l'administration des médicaments par nébulisation en ventilation mécanique devrait être optimisée en tenant compte des facteurs associés au dispositif de nébulisation, au circuit de ventilation et aux paramètres ventilatoires.[166] L'ensemble de ces facteurs modifie le degré de déposition de l'aérosol à l'intérieur du circuit de ventilation et par conséquent, affecte la dose inhalée ainsi que le dépôt pulmonaire.

Au cours de notre étude *in vitro*, le rendement de deux dispositifs de nébulisation a été évalué. Étant donné que le circuit de ventilation et les paramètres ventilatoires avaient été normalisés pour l'ensemble des expériences, la différence observée entre les doses inhalées moyennes obtenues avec le nébuliseur pneumatique (17%) et le nébuliseur à tamis vibrant (46%) repose uniquement sur la différence du type de dispositif utilisé. En effet, les caractéristiques intrinsèques (*i.e.*, volume de diluant, débit de nébulisation, positionnement du nébuliseur) sont propres au fonctionnement de chaque type de nébuliseur et dictent la dose émise, la répartition en taille des gouttelettes (MMAD) de l'aérosol et la dose inhalée (chapitre 5). Par conséquent, le respect des indications du fabricant est impératif pour une performance optimale du nébuliseur.

Puisque la milrinone est presque exclusivement éliminée par les reins (>95%), la dose inhalée chez les patients peut être déduite à partir de la quantité totale de médicament excrétée dans l'urine. Pour ce faire, nous avons mis au point une méthode analytique HPLC-UV permettant de déconjuguer la milrinone O-glucuronide et quantifier la milrinone totale excrétée dans l'urine suite à l'inhalation (annexe III, manuscrit n˚6 co-

premier auteur).[167] La différence observée entre les doses estimées par les mesures *in vitro* (46%) et *in vivo* (26% récupérée dans l'urine) avec le nébuliseur à tamis vibrant peut être expliquée par plusieurs variables.

D'abord, il s'agit d'un système fermé (*in vitro*) vs ouvert (*in vivo*). Dans le contexte des expériences en laboratoire, un filtre était placé à l'extrémité distale du tube endotrachéal pour collecter la dose inhalée *in vitro*. Or, la quantité de milrinone captée par le filtre à chaque phase inspiratoire du ventilateur y restait emprisonnée tout au long de la nébulisation. Dans un contexte clinique, *i.e.*, en absence de filtre, lors des phases inspiratoire et expiratoire du ventilateur les goutelettes en suspension formant le nuage d'aérosol sont libres de circuler de part et d'autre de l'endroit où aurait été placé le filtre (*in vitro*). Autrement dit, contrairement au système fermé (*in vitro*), dans un système ouvert (*in vivo*) plus représentatif de la réalité, une fraction des goutelettes inspirées qui atteignent l'extrémité distale du tube endotrachéal seront éventuellement expirées et non captées par le filtre (*in vitro*). Cette différence entre les deux systèmes a comme résultat d'entraîner une fraction expirée *in vivo* plus importante et par conséquent, une dose inhalée réduite. Or, cette fraction expirée et le dépôt pulmonaire dépendent de la répartition en taille des gouttelettes de l'aérosol qui, elle, est définie pour chaque type de dispositif de nébulisation (chapitre 5). Dans nos études (*in vitro* et *in vivo*), la dose inhalée mesurée représente la fraction déposée dans la région trachéobronchique et respiratoire des poumons du patient et correspond par définition à la dose inhalée respirable (*i.e.*, gouttelettes inhalées <5 μm).[105] Or, en ventilation mécanique, les goutelettes doivent avoir un diamètre aérodynamique médian en masse (MMAD) entre 1 et 3 μm,[95] voire inférieur à 2 μm[108-110] pour obtenir un dépôt pulmonaire profond. D'un autre côté, les goutelettes inférieures à 1 μm finissent éventuellement par être expirées à l'extérieur des poumons. En absence d'étude sur la déposition pulmonaire, nous n'avons pas pu connaître la fraction de la dose inhalée qui était inférieure à 2 μm. Cependant, lors d'une seconde étude *in vitro* s'inscrivant dans la continuité de celle-ci, notre laboratoire s'est intéressé à caractériser l'aérosol généré dans sa répartition en taille des gouttelettes afin de permettre la détermination de la fraction absorbée au niveau alvéolaire. Cette étude plus approfondie utilise les mêmes conditions cliniques en

y ajoutant un impacteur à cascade pour reproduire les différents niveaux de ramifications pulmonaires.

Une autre variable importante permettrait d'expliquer la différence observée entre les résultats *in vitro* et *in vivo* en terme de dose inhalée, soit une différence au niveau du circuit de ventilation. Contrairement aux expériences *in vitro*, la présence d'un humidificateur chauffant chez les patients est nécessaire pour éviter l'assèchement des voies aériennes et la lésion des muqueuses. Toutefois, cette hygrométrie artificielle entraîne une impaction plus importante de l'aérosol dans le circuit du ventilateur et plusieurs études *in vitro* ont démontré une diminution de la dose inhalée pouvant atteindre jusqu'à 50% selon le dispositif de nébulisation utilisé.[95, 98, 168] Puisqu'il est cliniquement déconseillé de contourner l'utilisation de l'humidificateur chauffant pour une durée dépassant 15 min,[97] une solution simple pour pallier à cette perte de médicament dans le circuit consiste à augmenter la dose nominale (dose de charge).

De plus, les paramètres ventilatoires (e.g., volume courant, débit et temps inspiratoire) n'étaient pas normalisés chez l'ensemble de nos patients et pouvaient être ajustés selon l'état respiratoire et le poids de chaque individu. D'ailleurs, ces facteurs peuvent être responsables d'une plus grande variabilité, tel qu'observé au niveau des résultats *in vivo* en général. Une manière d'augmenter la dose inhalée et la déposition pulmonaire d'un médicament chez les adultes soumis à la ventilation mécanique consiste à régler le volume courant à une valeur supérieure à 500 ml.[169] Autrement, le débit inspiratoire peut être diminué à 40-50 L/min[170, 171] en autant que ces paramètres puissent être compatibles avec l'état respiratoire du patient. Le débit inspiratoire étant inversement proportionnel à la dose inhalée, ce plus faible débit (e.g., comparativement à 80 L/min) permet de réduire la turbulence dans le circuit et l'impaction inertielle des particules d'aérosol.[172] Ainsi, certains réglages ont récemment été démontrés être associés à un meilleur rendement de la nébulisation en ventilation mécanique.[173, 174]

Finalement, étant donné qu'une collecte urinaire complète sur 24 h n'est pas toujours possible chez les patients avec la milrinone, la dose inhalée respirable doit être estimée dans le cadre des études pharmacocinétiques. Notre technique d'estimation consistait à soustraire, de la dose nominale (5 mg), l'ensemble des pertes de médicament dans les

systèmes de nébulisation et de ventilation. Certaines pertes étaient directement quantifiables *in vivo* (i.e., dose résiduelle, dose exhalée), alors que pour celles qui ne l'étaient pas, les résultats *in vitro* étaient substitués. Pour les raisons mentionnées dans le paragraphe précédent par rapport aux différences existant entre les expériences *in vitro* et *in vivo*, notre technique risque de surestimer la dose inhalée chez les patients. Cependant, lorsque nous l'avons appliquée chez les patients pour lesquels une collecte urinaire complète sur 24 h était disponible, la concordance ($P$=0.112) entre la dose inhalée moyenne estimée (30%) et la dose moyenne récupérée dans l'urine (26%) nous démontre que notre estimation est probablement exacte ou du moins très représentative de la réalité.

## 12.3. Caractérisation de la pharmacocinétique de la milrinone

### 12.3.1. Absorption de la milrinone suite à l'inhalation

Chez l'ensemble de nos patients, la portion ascendante de la courbe des concentrations en fonction du temps de la milrinone était bien caractérisée par une fonction d'entrée dans l'organisme limitée par la vitesse de nébulisation (ordre 0). En effet, comme dans le cas de plusieurs médicaments administrés par inhalation (chapitre 5), une absorption pulmonaire presque instantanée était anticipée pour la milrinone inhalée considérant ses propriétés physico-chimiques (PM, logP, pKa), le haut débit sanguin et l'énorme superficie des poumons.

### 12.3.2. Choix du modèle compartimental

La PK de la milrinone a déjà été étudiée chez les patients en chirurgie cardiaque sous CEC après administration intraveineuse.[91-94] Les première études[91, 92] n'avaient pas échantillonné assez longtemps pour caractériser adéquatement la demi-vie d'élimination du médicament. Deux études[93, 94] ont caractérisé le profil PK complet chez les patients en ayant opté pour un modèle à trois plutôt qu'à deux compartiments pour décrire leurs données regroupées et ce, en se basant sur le critère de Schwartz-Bayesian. Selon nous, la présence du troisième compartiment n'est pas tout à fait justifiée dans le contexte de leur étude. D'abord, la caractérisation de la troisième pente du profil PK des patients a été exécutée de manière sous-optimale, puisqu'elle n'était

basée que sur les deux derniers prélèvements. De plus, chez plusieurs patients les concentrations de ces deux échantillons se trouvaient inférieures à la limite de quantification de la méthode analytique utilisée.[93] Par ailleurs, les auteurs rapportent que, lorsque les analyses étaient effectuées sur les données individuelles, près de la moitié des patients étaient mieux caractérisés par le modèle à deux compartiments.[94] Chez la population de patients souffrant d'insuffisance cardiaque, la PK de la milrinone a plus souvent été décrite par un modèle à deux compartiments.[88, 90] Lors des analyses PK sur nos données individuelles, le modèle à deux compartiments décrivait plus adéquatement l'évolution temporelle des concentrations plasmatiques chez l'ensemble des patients. L'ajout d'un troisième compartiment n'a pas permis une meilleure caractérisation du profil PK de la milrinone selon le critère diagnostique d'Akaike.

Lorsqu'utilisée en chirurgie cardiaque, la CEC (et la réponse inflammatoire systémique qu'elle génère) peut jouer un rôle important dans la modification de la PK des médicaments administrés pendant la procédure.[175-178] Les composantes du circuit de CEC contribuent à l'altération du fonctionnement physiologique normal chez le patient et, par conséquent, affectent la façon dont les médicaments sont distribués et éliminés par l'organisme. Ces changements résultent de l'hémodilution,[179, 180] l'hypothermie,[181-183] l'hypotension et l'altération du débit sanguin des organes,[183, 184] l'isolement des poumons,[185, 186] et la séquestration du médicament dans le circuit de CEC.[187] L'impact de ces facteurs sur la PK de certains médicaments communément administrés durant la CEC a été étudié, notamment celle des inhibiteurs de la phosphodiestérase tels que l'amrinone et la milrinone. Contrairement à l'amrinone,[188] la milrinone reste minimalement ou pas liée au circuit de CEC.[189] Selon des résultats obtenus chez l'adulte, la CEC ne semble pas avoir de conséquence significative sur la PK de la milrinone.[91, 94] Cependant, une durée prolongée de la CEC risquerait d'affecter la fonction rénale du patient.[92]

Chez nos patients, aucune différence significative n'a été notée lorsque les concentrations plasmatiques étaient mesurées immédiatement (2 min) avant et après l'initiation ou le sevrage de la CEC. Dans une autre étude, les profils PK étaient

comparables chez les patients adultes ayant reçu la milrinone durant la CEC comparativement à ceux l'ayant reçu après la procédure.[94] En pédiatrie, certains ont suggéré d'ajouter un compartiment additionnel pour tenir compte de la composante CEC lors de la modélisation des données PK.[190] Toutefois, l'intégration d'une telle composante n'était pas jugée nécessaire dans notre cas. Ceci peut s'expliquer par le fait que, chez les patients cardiaques pédiatriques, l'effet de l'hémodilution est beaucoup plus important que chez l'adulte et, par conséquent, exerce un impact non-négligeable sur le volume de distribution. En effet, les volumes du circuit de CEC chez les patients pédiatriques ne peuvent pas être réduits proportionnellement à la diminution de leurs poids et taille comparativement à ceux de l'adulte, ce qui explique l'utilisation fréquente d'une ultrafiltration modifiée pour atténuer les effets de l'hémodilution.

Enfin, les paramètres PK estimés pour la milrinone inhalée chez nos patients avec un modèle simple à deux compartiments étaient comparables à ceux obtenus suite à l'analyse non-compartimentale ainsi qu'à ceux rapportés lors des études précédentes sur la milrinone intraveineuse administrée chez les patients en chirurgie cardiaque sous CEC[91, 92] et chez les patients souffrant d'insuffisance cardiaque.[81, 88, 90] Or, ceci suggère qu'un compartiment additionnel pour la composante CEC n'est pas nécessaire et démontre également que la dose inhalée que nous avons estimée est probablement exacte.

## 12.4. Caractérisation de la pharmacodynamie de la milrinone

Dans les études pharmacodynamiques, le choix du biomarqueur ressort comme un élément crucial permettant de suivre l'évolution temporelle de la réponse pharmacologique suite à l'administration d'un médicament. Plusieurs éléments doivent être considérés:[152]

- la pertinence du biomarqueur PD au mécanisme d'action du médicament (*i.e.*, plausibilité bilologique)
- le temps d'installation de l'effet du biomarqueur PD suite à l'administration du médicament

- la sensibilité du biomarqueur PD aux changements de concentration du médicament
- la fenêtre dynamique du biomarqueur PD associée à la fenêtre d'exposition au médicament
- la relation entre les changements au niveau du biomarqueur PD et l'issue clinique

Dans le cas de la milrinone, l'administration par voie inhalée ne représente pas encore une indication approuvée par la *U.S. Food and Drug Administration* (*FDA*) et *Santé Canada* pour le traitement de l'HP et, à notre connaissance, aucun biomarqueur PD n'a été clairement établi. La majorité des études cliniques[67-70, 75] portant sur la milrinone se sont servies des valeurs absolues de paramètres hémodynamiques, telles la résistance vasculaire pulmonaire (RVP) et la pression artérielle pulmonaire (PAP), pour étudier l'efficacité du médicament pour réduire les pressions pulmonaires suite au sevrage de la CEC en chirurgie cardiaque (chapitre 3). En procédant ainsi, l'effet reconnu de l'anesthésie générale sur les pressions pulmonaires[31] n'est pas discriminé de l'effet pharmacologique vasodilatateur anticipé pour la milrinone inhalée. Pour plusieurs raisons, le ratio PAm/PAPm apparaissait comme étant un choix de biomarqueur PD plus adapté dans le contexte de nos études cliniques (chapitre 2, manuscrit n˚1). Nous nous attendions à ce que l'effet de la milrinone inhalée puisse se manifester par une augmentation proportionnelle du ratio PAm/PAPm chez nos patients cardiaques sous anesthésie générale, un effet qui semble être supporté par les résultats observés lors d'études récentes que notre groupe a effectuées sur des médicaments inhalés avec un effet vasodilatateur.[71, 78, 156]

Les cliniciens procèdent généralement à la détermination du ratio PAm/PAPm à des moments prédéfinis (valeurs ponctuelles) avant l'inhalation pour déterminer la ligne de base ($R_0$) et suivant l'inhalation du médicament incluant post-inhalation ($R_{max}$) et post-CEC ($R_{post\text{-}CEC}$). Les ratios post- sont ensuite comparés à la valeur de base pour vérifier s'il y a différence significative ($\Delta_{Rmax\text{-}R0}$). Toutefois, cette évaluation des différences ne permet ni de caractériser adéquatement l'effet maximal ni de décrire l'évolution temporelle de l'effet du médicament. Dans le but de caractériser l'effet de la milrinone au cours de nos études, le ratio PAm/PAPm était continuellement enregistré tout au long

de l'intervention chirurgicale et ce, en plus des valeurs ponctuelles $R_0$, $R_{max}$, $R_{post\text{-}CEC}$ et $\Delta_{Rmax\text{-}R0}$. Par contre, notre plus grand obstacle résidait au niveau des manipulations chirurgicales. Bien que le ratio demeure inchangé sous l'effet de l'anesthésie, cela n'est plus le cas en présence de stress chirurgical. La plupart du temps, l'effet des manipulations chirurgicales exerçait une augmentation plus importante au niveau des pressions systémiques que des pressions pulmonaires. Par conséquent, cela pouvait engendrer une surestimation du ratio au moment de la prise de mesures des valeurs critiques et, dépendamment qu'il s'agissait du $R_0$ ou $R_{max}$, la valeur $\Delta_{Rmax\text{-}R0}$ risquait d'être sous-estimée ou surestimée, respectivement. Étant donné que $R_0$ représente une valeur de référence importante pour nos analyses, lorsque sa détermination risquait d'être imprécise à cause des manipulations chirurgicales inévitables durant la période pré-inhalation, nous devions nous référer à la valeur postopératoire (*i.e.*, retour à la ligne de base) afin d'éviter de sélectionner une valeur contaminée par l'artefact chirurgical en question. Les fluctuations du ratio PAm/PAPm observées immédiatement avant l'inhalation de la milrinone chez nos patients étaient principalement causées par les manipulations reliées à l'examen d'échographie transoesophagienne (ETO) qui devait être complété avant l'administration du médicament. La durée et l'intensité de cet examen pouvaient varier selon l'état de santé du patient et l'expérience de l'anesthésiologiste qui l'exécutait. Or, une manière d'éviter cet artefact aurait été d'avoir le même anesthésiste pour tous les cas d'intervention chirurgicale ou de laisser suffisamment de temps pour que le ratio puisse se stabiliser complètement avant l'inhalation. Malheureusement, cela n'était pas toujours possible puisque nous devions également nous assurer que l'inhalation (10-30 min) et les manipulations chirurgicales préparatoires soient terminées avant le début de la CEC. Enfin, il était aussi important de ne pas prolonger inutilement la durée de la procédure chirurgicale.

## 12.5. Choix de l'approche des aires sous la courbe pour l'analyse PK/PD

La caractérisation de la relation PK/PD de la milrinone inhalée s'est avérée problématique à cause des nombreuses interférences sur le ratio PAm/PAPm engendrées par les manipulations chirurgicales. En dépit du fait que nos données étaient riches, la

modélisation PK/PD des données individuelles de la relation concentration-effet, que ce soit par un lien direct ou avec l'ajout d'un compartiment effet s'est avérée impossible, par manque de convergence. Le recours à la pharmacocinétique de population n'a pas réglé le problème. En effet, comme les interférences pouvaient causer des fluctuations tant positives que négatives, il était impossible de procéder à un nettoyage des données sans risquer de les biaiser. Par conséquent, aucun modèle mathématique capable de prédire l'évolution temporelle de l'effet de la milrinone sur le ratio PAm/PAPm n'a pu être établi chez un patient donné.

Nous avons donc opté pour l'approche des aires sous la courbe afin de déterminer une exposition à l'effet net sans devoir dissocier arbitrairement les effets causés par les artéfacts chirurgicaux. Nos analyses se sont concentrées sur la relation concentration-effet durant la période d'inhalation (*i.e.*, 0 min à fin de nébulisation), puisque la majorité des manipulations chirurgicales survenaient normalement plus tard durant l'intervention, voire lors des préparations pour la procédure extracorporelle (*e.g.* points en bourse, cannulations artérielle et veineuse, etc.). De plus, la période définie par l'inhalation (i.e., 0 min à fin d'inhalation) représente l'espace temporel au cours duquel l'effet direct de la milrinone pouvait être nettement observé. Nous avons également effectué les analyses pour la période pré-CEC (i.e., 0 min à début de CEC), mais aucune corrélation significative n'a pu être notée en appui de la présence d'un effet soutenu pour la milrinone inhalée. Ceci peut, en partie, être expliqué par une plus grande contamination de l'effet causée par la présence accrue des manipulations reliées à la préparation de la CEC durant cette période.

Durant nos analyses, nous avons supposé que les patients étaient tous soumis au même stress chirurgical pendant la période étudiée. En réalité, ceci n'était pas toujours le cas puisque certains patients avaient une fin de nébulisation qui coïncidait avec le début de la CEC. Autrement dit, la caractérisation de l'effet net chez ces patients pourrait avoir été surestimée due à la présence de davantage d'artéfacts chirurgicaux comparativement aux patients chez lesquels plus de temps se serait écoulé entre la fin de l'inhalation et le début de la CEC. De manière générale, les manipulations chirurgicales avaient des répercussions plus importantes sur les pressions artérielles systémiques que pulmonaires

se traduisant par une augmentation nette de la PAm; ce qui, par conséquence, contribuait à l'augmentation de l'ASCE du ratio PAm/PAPm. Or, le rythme de l'intervention, la durée et l'intensité de chaque manipulation sont des facteurs qui dépendent beaucoup de la maîtrise des techniques de travail du chirurgien en charge et qui contribuent à influencer l'amplitude et la durée de l'interférence sur la caractérisation de l'effet de la milrinone. Toutefois, exiger que toutes les interventions chirurgicales du projet soient exécutées par le même chirurgien n'aurait pas été une solution réaliste, puisque cela aurait entraîné un prolongement excessif de la durée totale des études cliniques.

Outre la présence de manipulations chirurgicales, une raison pouvant expliquer la fréquence accrue de fluctuations du ratio PAm/PAPm chez nos patients est reliée à leur état de santé. Les patients chez lesquels nous avons étudié la relation PK/PD de la milrinone ont été recrutés durant la même période de recrutement que les patients d'une étude concomitante menée à notre centre et randomisée pour la milrinone inhalée. Lors du recrutement, les patients dont l'hypertension pulmonaire était plus sévère étaient préférentiellement sélectionnés pour notre étude afin qu'ils reçoivent la milrinone inhalée en prophylaxie au lieu d'être potentiellement randomisés dans le groupe placebo de l'autre étude. Or, ce biais de sélection peut expliquer une plus grande vulnérabilité à l'instabilité hémodynamique chez nos patients, ainsi qu'une représentativité moins juste de la population de patients en chirurgie cardiaque chez qui le traitement est normalement administré, rendant ainsi les résultats moins généralisables.

Une étude pédiatrique en chirurgie cardiaque qui utilisait la PAm comme biomarqueur PD pour établir la relation PK/PD du vasoconstricteur dexmedetomidine a également rapporté le problème d'artéfacts chirurgicaux (*i.e.*, effet de succion du tube endotrachéal) interférant avec la caractérisation de l'effet pharmacologique du médicament.[191] La méthode qu'ils proposent pour modéliser les artéfacts consiste à supposer qu'à chaque manipulation chirurgicale correspond une réponse physiologique provoquée par une poussée de noradrénaline libérée au niveau des nerfs sympathiques du patient. Ces « réponses physiologiques » sont ensuite modélisées comme des bolus de noradrénaline administrés dans un compartiment effet dont les vitesses d'entrée et de sortie (ordre 1) sont estimées comme des paramètres. De plus, ils supposent que les

concentrations du vasopresseur provoquent une augmentation immédiate de la PAm et que cette relation est décrite par une relation linéaire directe. La réponse observée au niveau de la PAm est alors considérée comme étant la somme de l'effet du médicament et de la « réponse physiologique » provoquée par la manipulation chirurgicale.

Au cours de nos études, le moment et la durée de chacune des manipulations chirurgicales n'ont pas nécessairement été identifiés et compilés à des fins d'analyse. Chez l'adulte, l'inhalation des médicaments, les prélèvements plasmatiques et la collecte des données hémodynamiques se déroulent tous du côté de la tête du patient, isolé du champ opératoire stérile. Par conséquent, avant l'arrivée des caméras vidéo dans les salles d'opération, il nous aurait fallu attribuer la tâche de rapporter l'heure et la nature des différentes manipulations chirurgicales à un autre membre de l'équipe de recherche. Il était donc difficile d'utiliser la technique de Potts *et al.* pour associer, de manière systématique, toutes les fluctuations du ratio PAm/PAPm supérieures à 20% à des artéfacts chirurgicaux, surtout pour celles observées à la fin de la période d'inhalation. De plus, cette méthode ne permet pas de prendre en considération les artéfacts qui avaient un effet négatif, i.e., une diminution de la PAm.

Étant donné qu'il est pratiquement impossible d'éviter la présence d'artéfacts chirurgicaux, une façon de contrôler ce facteur confondant aurait été d'avoir un groupe placebo pour lequel des données PD auraient été collectées et analysées en comparaison avec celles du groupe milrinone inhalée. Dans une étude clinique pilote menée par le Dr. André Y. Denault,[71] l'évolution des paramètres hémodynamiques a été suivie pendant une procédure chirurgicale avec et sans milrinone inhalée. Toutefois, la collecte de données était très limitée, i.e., seulement cinq mesures ont été prises dont aucune durant la période d'inhalation. Dans cette étude, aucune différence significative n'a été notée en ce qui concerne l'évolution de la PAm, de la PAPm ou du ratio dans les deux groupes. Lors d'une étude animale initiée par notre laboratoire (en cours), l'effet de la milrinone sur le ratio PAm/PAPm sera caractérisé suite à une collecte de données riches en absence de manipulations chirurgicales durant et après la période d'inhalation. Ceci permettra d'obtenir une estimation non biaisée de l'effet pharmacologique réel chez un modèle porcin avec hypertension pulmonaire.

## 12.6. Modèle de régression logistique pour la sortie de circulation extracorporelle difficile

En médecine, la régression logistique constitue une méthode de choix pour rechercher et déterminer les facteurs de risque d'une maladie. L'intérêt d'une telle approche consiste à permettre la mise en place de mesures préventives et d'assurer une prise en charge ainsi qu'un suivi adaptés aux patients. Dans notre étude, l'utilisation d'une régression logistique visait à modéliser la probabilité d'observer une sortie de CEC difficile suivant l'administration prophylactique de la milrinone inhalée chez les patients subissant une chirurgie cardiaque. Plusieurs variables explicatives ont été testées lors de l'analyse univariée et seulement les variables pronostiques qui se sont avérées statistiquement significatives ont été retenues pour l'analyse multivariée. On entend par variable pronostique une variable qui peut être obtenue avant la CEC, *e.g.*, $R_0$, euroSCORE, ASCE, $R_{max}$, $\Delta_{Rmax-R0}$, $\Delta\%_{Rmax-R0}$. L'objectif étant d'identifier les variables ayant une valeur prédictive potentielle sur l'issue clinique, toutes celles recueillies au cours de l'étude n'étaient donc pas systématiquement testées dans la régression logistique. Notre sélection des variables a été fondée sur leur pertinence clinique et nécessite une connaissance préalable de la physiopathologie reliée à la CEC et des facteurs pouvant l'influencer. Ainsi, les variables susceptibles d'influencer la survenue ou non d'une sortie de CEC difficile pouvaient être reliées à l'état de santé du patient (*i.e.*, sévérité de l'HP et euroSCORE), à l'intervention chirurgicale (*i.e.*, durée de la CEC) ou à la réponse au traitement pharmacologique (*i.e.*, effet de la milrinone inhalée sur le ratio PAm/PAPm). Les variables sélectionnées pour notre modèle final sont la durée de CEC et $\Delta_{Rmax-R0}$.

Sur le plan technique, une limite importante serait le fait que nous ne disposons pas d'un échantillon suffisamment grand pour pouvoir justifier la présence des deux variables explicatives dans le modèle. En effet, la règle générale est d'avoir au moins dix fois plus d'événements que de variables explicatives incluses dans le modèle final.[192] Or, dans notre cas, le nombre d'événements observés pour la variable dépendante (n=10 sorties de CEC difficiles) serait insuffisant par rapport au nombre de variables explicatives conservées (n=2 variables). Par contre, notre étude consistait en une étude exploratoire

et il n'y a généralement pas de calcul de taille d'échantillon pour ce type d'études. Par ailleurs, l'objectif primaire de cette étude était de caractériser la relation PK/PD de la milrinone inhalée, donc notre analyse par régression logistique se voulait plutôt être une démarche d'ordre exploratoire et non confirmatoire visant à évaluer l'existence d'une association entre la réponse pharmacologique mesurée par notre biomarqueur PD et l'issue clinique. Ainsi, malgré la limite de taille d'échantillon, une corrélation significative a tout de même pu être démontrée entre $\Delta_{Rmax\text{-}R0}$ et la sortie de CEC difficile. Des futures études de plus grande envergure permettraient de confirmer, voire d'identifier d'autres variables permettant de mieux prédire la sortie de CEC difficile chez les patients subissant une chirurgie cardiaque.

Bien que les analyses ne soient pas encore complétées, une étude rétrospective menée à l'ICM sur l'effet préventif de la milrinone inhalée administrée en chirurgie cardiaque avait également pour objectif de déterminer les variables explicatives du phénomène de sortie de CEC difficile. Le même type d'analyses statistiques a été appliqué sur une banque de données de 101 patients incluant l'éventail complet des variables reliées à l'état de santé du patient, au type d'intervention chirurgicale et à la CEC. Cette analyse nous permettra de tester l'ensemble des facteurs de risque plausibles en chirurgie cardiaque et d'avoir un nombre suffisant d'événements pour justifier la présence de plusieurs variables explicatives. De plus, comme pour tout test statistique, il conviendra de vérifier certaines conditions d'application afin de confirmer la validité et la pertinence du modèle de régression logistique: colinéarité entre les variables explicatives, robustesse et adéquation du modèle. Les résultats préliminaires de cette étude suggèrent également que la durée de CEC soit considérée comme un facteur de risque potentiel de la sortie de CEC difficile.

# Conclusion et perspectives

Appliquée au domaine de la chirurgie cardiaque, l'utilisation de médicaments administrés par voie pulmonaire (inhalation) chez les patients sous ventilation mécanique a énormément évoluée au cours des dernières années. Nos études cliniques exploratoires (phase IIa) consistaient à évaluer, suite à l'estimation de la dose inhalée et l'établissement du modèle PK, l'effet de la milrinone inhalée sur le ratio PAm/PAPm. Malgré le fait que, pour diverses raisons, un modèle PK/PD n'ait pu être développé pour en établir la preuve de concept, une relation linéaire significative entre l'exposition et la réponse a tout de même été démontrée en utilisant l'approche des aires sous la courbe (ASCE vs ASC).

Il n'a pas encore été démontré de manière empirique que l'effet de la milrinone inhalée sur le ratio PAm/PAPm conduise directement à la prévention d'une sortie de CEC difficile (issue clinique). Par contre, la défaillance du ventricule droit (conséquence la plus redoutée de l'HP) a été démontrée être directement associée à une augmentation de la mortalité chez les patients subissant une chirurgie cardiaque. D'autre part, la démonstration d'une relation causale nécessite que le biomarqueur soit déjà validé comme paramètre substitut. En effet, cette confirmation causale est peu probable lorsque le lien entre le biomarqueur et l'issue clinique n'a pas été clairement établi, comme c'est souvent le cas pour une nouvelle indication ou nouvelle voie d'administration. Après avoir vérifié l'existence d'une corrélation entre le ratio PAm/PAPm (biomarqueur PD) et la sortie de CEC difficile (issue clinique), nous proposons la mesure ponctuelle du ratio PAm/PAPm, i.e., $\Delta_{Rmax\text{-}R0}$, comme candidat pour devenir un biomarqueur substitut puisqu'il serait beaucoup plus facile de le mesurer dans une étude prospective de grande envergure.

Les travaux de cette thèse ont permis de mieux comprendre les enjeux reliés au développement de nouveaux médicaments administrés par inhalation dans le contexte de la chirurgie cardiaque. Une corrélation a été démontrée entre l'exposition aux concentrations systémiques de la milrinone et son effet pharmacologique suite à son administration par inhalation et ce, en utilisant le ratio PAm/PAPm comme biomarqueur

PD. Une mesure ponctuelle de ce biomarqueur lors de l'effet maximal corrèle également avec l'issue clinique. Ce biomarqueur pourrait donc, une fois validé, être utilisé comme paramètre substitut en chirurgie cardiaque pour démontrer l'efficacité de la milrinone inhalée dans la prévention d'une sortie de CEC difficile. Ultimement, l'inclusion d'une composante PK/PD au niveau de futures études de phase III sur la milrinone inhalée permettrait de mieux prédire l'impact d'un changement de dose sur la durée d'action du médicament et, par conséquent, proposer un régime posologique optimal afin d'améliorer l'utilisation clinique de cet agent vasoactif reconnu pour son efficacité et sa faible toxicité.

# Bibliographie


1. Statistics Canada. *Mortality, Summary List of Causes 2009*. 2012; Available from: http://www.statcan.gc.ca/pub/84f0209x/84f0209x2009000-eng.htm.
2. Statistique Canada. *Projections démographiques pour le Canada, les provinces et les territoires (91-520-X), 2009 à 2036, scénario croissance moyenne (M1)*. 2010; Available from: http://www5.statcan.gc.ca/olc-cel/olc.action?ObjId=91-520-X&ObjType=2&lang=fr&limit=0.
3. Machin, D. and C. Allsager, *Principles of cardiopulmonary bypass.* Continuing Education in Anaesthesia, Critical Care & Pain, 2006. **6**(5): p. 176-181.
4. Wan, S., J.L. LeClerc, and J.L. Vincent, *Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies.* Chest, 1997. **112**(3): p. 676-692.
5. El Kebir, D., et al., *Effects of inhaled nitric oxide on inflammation and apoptosis after cardiopulmonary bypass.* Chest, 2005. **128**(4): p. 2910-7.
6. McMullan, D.M., et al., *Alterations in endogenous nitric oxide production after cardiopulmonary bypass in lambs with normal and increased pulmonary blood flow.* Circulation, 2000. **102**(19 Suppl 3): p. Iii172-8.
7. Kirshbom, P.M., et al., *Effects of cardiopulmonary bypass and circulatory arrest on endothelium-dependent vasodilation in the lung.* J Thorac Cardiovasc Surg, 1996. **111**(6): p. 1248-56.
8. Morita, K., et al., *Pulmonary vasoconstriction due to impaired nitric oxide production after cardiopulmonary bypass.* Ann Thorac Surg, 1996. **61**(6): p. 1775-80.
9. Seghaye, M.C., et al., *Endogenous nitric oxide production and atrial natriuretic peptide biological activity in infants undergoing cardiac operations.* Crit Care Med, 1997. **25**(6): p. 1063-70.
10. Lamarche, Y., et al., *Inhaled but not intravenous milrinone prevents pulmonary endothelial dysfunction after cardiopulmonary bypass.* J.Thorac.Cardiovasc.Surg., 2005. **130**(1): p. 83-92.
11. Garzon, A.A., B. Seltzer, and K.E. Karlson, *Respiratory mechanics following open-heart surgery for acquired valvular disease.* Circulation, 1966. **33**(4 Suppl): p. I57-64.
12. Lesage, A.M., et al., *Pathogenesis of pulmonary damage during extracorporeal perfusion.* Arch Surg, 1966. **93**(6): p. 1002-8.
13. Shimizu, T. and F.J. Lewis, *An experimental study of pulmonary function following cardiopulmonary bypass.* J Thorac Cardiovasc Surg, 1966. **52**(4): p. 565-70.

14. Garzon, A.A., et al., *Influence of open-heart surgery on respiratory work.* Dis Chest, 1967. **52**(3): p. 392-6.

15. Ghia, J. and N.B. Andersen, *Pulmonary function and cardiopulmonary bypass.* Jama, 1970. **212**(4): p. 593-7.

16. Andersen, N.B. and J. Ghia, *Pulmonary function, cardiac status, and postoperative course in relation to cardiopulmonary bypass.* J Thorac Cardiovasc Surg, 1970. **59**(4): p. 474-83.

17. Braun, S.R., M.L. Birnbaum, and P.S. Chopra, *Pre- and postoperative pulmonary function abnormalities in coronary artery revascularization surgery.* Chest, 1978. **73**(3): p. 316-20.

18. Shenkman, Z., et al., *The effects of cardiac surgery on early and late pulmonary functions.* Acta Anaesthesiol Scand, 1997. **41**(9): p. 1193-9.

19. Locke, T.J., et al., *Rib cage mechanics after median sternotomy.* Thorax, 1990. **45**(6): p. 465-8.

20. Matte, P., et al., *Effects of conventional physiotherapy, continuous positive airway pressure and non-invasive ventilatory support with bilevel positive airway pressure after coronary artery bypass grafting.* Acta Anaesthesiol Scand, 2000. **44**(1): p. 75-81.

21. Robitaille, A., et al., *Importance of relative pulmonary hypertension in cardiac surgery: the mean systemic-to-pulmonary artery pressure ratio.* J Cardiothorac Vasc Anesth, 2006. **20**(3): p. 331-9.

22. Denault, A.Y., et al., *The importance of difficult separation from cardiopulmonary bypass: the Montreal and Quebec heart institute experience.* Experimental and clinical cardiology, 2006. **11**(1): p. 37-38.

23. Salem, R., et al., *Left ventricular end-diastolic pressure is a predictor of mortality in cardiac surgery independently of left ventricular ejection fraction.* Br J Anaesth, 2006. **97**(3): p. 292-7.

24. Denault, A.Y., et al., *Difficult and complex separation from cardiopulmonary bypass in high-risk cardiac surgical patients: a multicenter study.* J Cardiothorac Vasc Anesth, 2012. **26**(4): p. 608-16.

25. Tuman, K.J., et al., *Morbidity and duration of ICU stay after cardiac surgery. A model for preoperative risk assessment.* Chest, 1992. **102**(1): p. 36-44.

26. Malouf, J.F., et al., *Severe pulmonary hypertension in patients with severe aortic valve stenosis: clinical profile and prognostic implications.* J Am Coll Cardiol, 2002. **40**(4): p. 789-95.

27. Carricart, M., et al., *Incidence and significance of abnormal hepatic venous Doppler flow velocities before cardiac surgery.* J Cardiothorac Vasc Anesth, 2005. **19**(6): p. 751-8.

28. Davila-Roman, V.G., et al., *Right ventricular dysfunction in low output syndrome after cardiac operations: assessment by transesophageal echocardiography.* Ann Thorac Surg, 1995. **60**(4): p. 1081-6.

29. Denault, A.Y., et al., *Tezosentan and right ventricular failure in patients with pulmonary hypertension undergoing cardiac surgery: the TACTICS trial.* J Cardiothorac Vasc Anesth, 2013. **27**(6): p. 1212-7.

30. Gomez, C.M. and M.G. Palazzo, *Pulmonary artery catheterization in anaesthesia and intensive care.* Br.J.Anaesth., 1998. **81**(6): p. 945-956.

31. Robitaille, A., et al., *Importance of relative pulmonary hypertension in cardiac surgery: the mean systemic-to-pulmonary artery pressure ratio.* J Cardiothorac Vasc Anesth, 2006. **20**(3): p. 331-339.

32. Therrien, J., et al., *CCS Consensus Conference 2001 update: recommendations for the management of adults with congenital heart disease. Part I.* Can.J.Cardiol., 2001. **17**(9): p. 940-959.

33. Therrien, J., et al., *Canadian Cardiovascular Society Consensus Conference 2001 update: Recommendations for the Management of Adults with Congenital Heart Disease--Part II.* Can.J.Cardiol., 2001. **17**(10): p. 1029-1050.

34. Simonneau, G., et al., *Updated clinical classification of pulmonary hypertension.* J.Am.Coll.Cardiol., 2009. **54**(1 Suppl): p. S43-S54.

35. Downing, S.W. and L.H. Edmunds, Jr., *Release of vasoactive substances during cardiopulmonary bypass.* Ann.Thorac.Surg., 1992. **54**(6): p. 1236-1243.

36. Asimakopoulos, G., et al., *Lung injury and acute respiratory distress syndrome after cardiopulmonary bypass.* Ann.Thorac.Surg., 1999. **68**(3): p. 1107-1115.

37. Lesage, A.M., et al., *Pathogenesis of pulmonary damage during extracorporeal perfusion.* Arch.Surg., 1966. **93**(6): p. 1002-1008.

38. Kaul, T.K. and B.L. Fields, *Postoperative acute refractory right ventricular failure: incidence, pathogenesis, management and prognosis.* Cardiovasc.Surg., 2000. **8**(1): p. 1-9.

39. Hache, M., et al., *Inhaled prostacyclin (PGI2) is an effective addition to the treatment of pulmonary hypertension and hypoxia in the operating room and intensive care unit.* Can.J.Anaesth., 2001. **48**(9): p. 924-929.

40. Hache, M., et al., *Inhaled epoprostenol (prostacyclin) and pulmonary hypertension before cardiac surgery.* J.Thorac.Cardiovasc.Surg., 2003. **125**(3): p. 642-649.

41. Lamarche, Y., et al., *Preliminary experience with inhaled milrinone in cardiac surgery.* Eur.J Cardiothorac Surg., 2007. **31**(6): p. 1081-1087.

42. Tremblay, N.A., et al., *A simple classification of the risk in cardiac surgery: the first decade.* Can.J.Anaesth., 1993. **40**(2): p. 103-111.

43. Reich, D.L., et al., *Intraoperative hemodynamic predictors of mortality, stroke, and myocardial infarction after coronary artery bypass surgery.* Anesth.Analg., 1999. **89**(4): p. 814-822.

44. Bernstein, A.D. and V. Parsonnet, *Bedside estimation of risk as an aid for decision-making in cardiac surgery.* Ann.Thorac.Surg., 2000. **69**(3): p. 823-828.

45. Malouf, J.F., et al., *Severe pulmonary hypertension in patients with severe aortic valve stenosis: clinical profile and prognostic implications.* J.Am.Coll.Cardiol., 2002. **40**(4): p. 789-795.

46. Nilsson, J., et al., *Comparison of 19 pre-operative risk stratification models in open-heart surgery.* Eur.Heart J., 2006. **27**(7): p. 867-874.

47. D'Alonzo, G.E., et al., *Survival in patients with primary pulmonary hypertension. Results from a national prospective registry.* Ann.Intern.Med., 1991. **115**(5): p. 343-349.

48. Yeo, T.C., et al., *Value of a Doppler-derived index combining systolic and diastolic time intervals in predicting outcome in primary pulmonary hypertension.* Am.J Cardiol., 1998. **81**(9): p. 1157-1161.

49. Ramakrishna, G., et al., *Impact of pulmonary hypertension on the outcomes of noncardiac surgery: predictors of perioperative morbidity and mortality.* J Am.Coll.Cardiol., 2005. **45**(10): p. 1691-1699.

50. Voelkel, N.F., et al., *Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure.* Circulation, 2006. **114**(17): p. 1883-1891.

51. Haddad, F., et al., *The right ventricle in cardiac surgery, a perioperative perspective: II. Pathophysiology, clinical importance, and management.* Anesth Analg., 2009. **108**(2): p. 422-433.

52. Boldt, J., et al., *Right ventricular function in patients with aortic stenosis undergoing aortic valve replacement.* J.Cardiothorac.Vasc.Anesth., 1992. **6**(3): p. 287-291.

53. Davila-Roman, V.G., et al., *Right ventricular dysfunction in low output syndrome after cardiac operations: assessment by transesophageal echocardiography.* Ann.Thorac.Surg., 1995. **60**(4): p. 1081-1086.

54. Fortier, S., et al., *Inhaled prostacyclin reduces cardiopulmonary bypass-induced pulmonary endothelial dysfunction via increased cyclic adenosine monophosphate levels.* J.Thorac.Cardiovasc.Surg., 2004. **128**(1): p. 109-116.

55. Doolan, L.A., et al., *A placebo-controlled trial verifying the efficacy of milrinone in weaning high-risk patients from cardiopulmonary bypass.* J Cardiothorac Vasc Anesth, 1997. **11**(1): p. 37-41.

56. Kikura, M., et al., *The effects of milrinone on platelets in patients undergoing cardiac surgery.* Anesth Analg, 1995. **81**(1): p. 44-8.

57. Baim, D.S., et al., *Evaluation of a new bipyridine inotropic agent--milrinone--in patients with severe congestive heart failure.* N Engl J Med, 1983. **309**(13): p. 748-56.

58. Denault, A.Y., et al., *Inhaled milrinone: a new alternative in cardiac surgery?* Semin Cardiothorac Vasc Anesth, 2006. **10**(4): p. 346-60.

59. Woolfrey, S.G., et al., *Dose regimen adjustment for milrinone in congestive heart failure patients with moderate and severe renal failure.* J Pharm Pharmacol, 1995. **47**(8): p. 651-5.

60. Overgaard, C.B. and V. Dzavik, *Inotropes and vasopressors: review of physiology and clinical use in cardiovascular disease.* Circulation, 2008. **118**(10): p. 1047-56.

61. Lobato, E.B., O. Florete, Jr., and H.L. Bingham, *A single dose of milrinone facilitates separation from cardiopulmonary bypass in patients with pre-existing left ventricular dysfunction.* Br J Anaesth, 1998. **81**(5): p. 782-4.

62. Kim, J.H., et al., *Prophylactic milrinone during OPCAB of posterior vessels: implication in angina patients taking beta-blockers.* Eur J Cardiothorac Surg, 2003. **24**(5): p. 770-6.

63. Hardy, J.F. and S. Belisle, *Inotropic support of the heart that fails to successfully wean from cardiopulmonary bypass: the Montreal Heart Institute experience.* J Cardiothorac Vasc Anesth, 1993. **7**(4 Suppl 2): p. 33-9.

64. Denault, A.Y., et al., *Dynamic right ventricular outflow tract obstruction in cardiac surgery.* J Thorac Cardiovasc Surg, 2006. **132**(1): p. 43-9.

65. Yamada, T., et al., *Hemodynamic effects of milrinone during weaning from cardiopulmonary bypass: comparison of patients with a low and high prebypass cardiac index.* J Cardiothorac Vasc Anesth, 2000. **14**(4): p. 367-73.

66. Solina, A., et al., *A comparison of inhaled nitric oxide and milrinone for the treatment of pulmonary hypertension in adult cardiac surgery patients.* J Cardiothorac Vasc Anesth, 2000. **14**(1): p. 12-7.

67. Haraldsson, A., N. Kieler-Jensen, and S.E. Ricksten, *The additive pulmonary vasodilatory effects of inhaled prostacyclin and inhaled milrinone in postcardiac surgical patients with pulmonary hypertension.* Anesth Analg, 2001. **93**(6): p. 1439-45.

68. Sablotzki, A., et al., *Selective pulmonary vasodilation with inhaled aerosolized milrinone in heart transplant candidates.* Can J Anaesth, 2005. **52**(10): p. 1076-82.

69. Wang, H., et al., *Comparison of inhaled and intravenous milrinone in patients with pulmonary hypertension undergoing mitral valve surgery.* Adv Ther, 2009. **26**(4): p. 462-8.

70. Hegazy, N. and A. Elhenawy, *Comparison of hemodynamic effects of inhaled milrinone and inhaled prostacyclin after adult cardiac surgery.* Journal of Applied Sciences Research, 2010. **6**(1): p. 38-44.

71. Denault, A., et al., *Pilot randomized controlled trial of inhaled milrinone in high-risk cardiac surgical patients.* Surgery Curr Res, 2014. **4**(4): p. 192.

72. Lamarche, Y., et al., *Preliminary experience with inhaled milrinone in cardiac surgery.* Eur J Cardiothorac Surg, 2007. **31**(6): p. 1081-7.

73. Guo, H.W., et al., *Effect of inhaling specific phosphodiesterase inhibitor on lung injury induced by cardiopulmonary bypass.* Zhonghua Yi Xue Za Zhi, 2011. **91**(20): p. 1401-4.

74. Gong, M., et al., *Preoperative inhalation of milrinone attenuates inflammation in patients undergoing cardiac surgery with cardiopulmonary bypass.* Med Princ Pract, 2012. **21**(1): p. 30-5.

75. Singh, R., et al., *Inhaled nitroglycerin versus inhaled milrinone in children with congenital heart disease suffering from pulmonary artery hypertension.* J Cardiothorac Vasc Anesth, 2010. **24**(5): p. 797-801.

76. Buckley, M.S. and J.P. Feldman, *Nebulized milrinone use in a pulmonary hypertensive crisis.* Pharmacotherapy, 2007. **27**(12): p. 1763-6.

77. Carev, M., et al., *Combined usage of inhaled and intravenous milrinone in pulmonary hypertension after heart valve surgery.* Coll Antropol, 2010. **34**(3): p. 1113-7.

78. St-Pierre, P., et al., *Inhaled milrinone and epoprostenol in a patient with severe pulmonary hypertension, right ventricular failure, and reduced baseline brain saturation value from a left atrial myxoma.* J Cardiothorac Vasc Anesth, 2014. **28**(3): p. 723-9.

79. Wagner, J.G., *Fundamentals of Clinical Pharmacokinetics*. 1975, Hamilton, Illinois: Drug Intelligence Publications.

80. Gibaldi, M. and D. Perrier, *Pharmacokinetics*. 2nd ed. 1982, New York: Marcel Dekker.

81. Stroshane, R.M., et al., *Oral and intravenous pharmacokinetics of milrinone in human volunteers.* J Pharm Sci, 1984. **73**(10): p. 1438-41.

82. Larsson, R., et al., *Pharmacokinetics and effects on blood pressure of a single oral dose of milrinone in healthy subjects and in patients with renal impairment.* Eur J Clin Pharmacol, 1986. **29**(5): p. 549-53.

83. Stroshane, R., D. Benziger, and J. Edelson, *Pharmacokinetics of milrinone in congestive heart failure patients.* Milrinone: investigation of new inotropic therapy for congestive heart failure. Raven Press, New York, 1984: p. 119-131.

84. Nanimatsu, H., et al., *Hemodynamic effects of milrinone in patients with congestive heart failure--short- and long-term follow up studies.* Jpn Circ J, 1993. **57**(2): p. 91-101.

85. Kubo, S.H., et al., *Acute dose range study of milrinone in congestive heart failure.* Am J Cardiol, 1985. **55**(6): p. 726-30.

86. Packer, M., et al., *Effect of oral milrinone on mortality in severe chronic heart failure. The PROMISE Study Research Group.* N Engl J Med, 1991. **325**(21): p. 1468-75.

87. Anderson, J.L., et al., *Efficacy and safety of sustained (48 hour) intravenous infusions of milrinone in patients with severe congestive heart failure: a multicenter study.* J Am Coll Cardiol, 1987. **9**(4): p. 711-22.

88. Edelson, J., et al., *Pharmacokinetics of the bipyridines amrinone and milrinone.* Circulation, 1986. **73**(3 Pt 2): p. III145-52.

89. Wilson, H., et al., *The pharmacokinetics of milrinone in patients with chronic cardiac failure.* Clinical Pharmacology and Therapeutics, 1984. **35**(2): p. 283-283.

90. Benotti, J.R., et al., *Pharmacokinetics and pharmacodynamics of milrinone in chronic congestive heart failure.* Am J Cardiol, 1985. **56**(10): p. 685-9.

91. Das, P.A., et al., *Disposition of milrinone in patients after cardiac surgery.* Br J Anaesth, 1994. **72**(4): p. 426-9.

92. De Hert, S.G., et al., *Comparison of two different loading doses of milrinone for weaning from cardiopulmonary bypass.* J Cardiothorac Vasc Anesth, 1995. **9**(3): p. 264-71.

93. Butterworth, J.F., et al., *A pharmacokinetic and pharmacodynamic evaluation of milrinone in adults undergoing cardiac surgery.* Anesth Analg, 1995. **81**(4): p. 783-92.

94. Bailey, J.M., et al., *Pharmacokinetics of intravenous milrinone in patients undergoing cardiac surgery.* Anesthesiology, 1994. **81**(3): p. 616-22.

95. Dhand, R. and M.J. Tobin, *Inhaled bronchodilator therapy in mechanically ventilated patients.* Am J Respir Crit Care Med, 1997. **156**(1): p. 3-10.

96. Dhand, R., *Inhalation therapy with metered-dose inhalers and dry powder inhalers in mechanically ventilated patients.* Respir Care, 2005. **50**(10): p. 1331-4; discussion 1344-5.

97. Dhand, R. and V.P. Guntur, *How best to deliver aerosol medications to mechanically ventilated patients.* Clin Chest Med, 2008. **29**(2): p. 277-96, vi.

98. Dhand, R. and E. Mercier, *Effective inhaled drug administration to mechanically ventilated patients.* Expert Opin Drug Deliv, 2007. **4**(1): p. 47-61.

99. Duarte, A.G., K. Momii, and A. Bidani, *Bronchodilator therapy with metered-dose inhaler and spacer versus nebulizer in mechanically ventilated patients: comparison of magnitude and duration of response.* Respir Care, 2000. **45**(7): p. 817-23.

100. Gay, P.C., et al., *Metered dose inhalers for bronchodilator delivery in intubated, mechanically ventilated patients.* Chest, 1991. **99**(1): p. 66-71.

101. Guerin, C., et al., *Inhaled fenoterol-ipratropium bromide in mechanically ventilated patients with chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 1999. **159**(4 Pt 1): p. 1036-42.

102. Byron, P.R., *Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation.* J Pharm Sci, 1986. **75**(5): p. 433-8.

103. Ari, A., *Jet, Ultrasonic, and Mesh Nebulizers An Evaluation of Nebulizers for Better Clinical Outcomes.* Euras J Pulm, 2014. **16**(1): p. 1-7.

104. Smaldone, G.C., *Drug delivery via aerosol systems: concept of "aerosol inhaled".* J Aerosol Med, 1991. **4**(3): p. 229-35.

105. Laube, B.L., *In vivo measurements of aerosol dose and distribution: clinical relevance.* J Aerosol Med, 1996. **9 Suppl 1**: p. S77-91.

106. Hess, D., et al., *Medication nebulizer performance. Effects of diluent volume, nebulizer flow, and nebulizer brand.* Chest, 1996. **110**(2): p. 498-505.

107. Patton, J.S. and P.R. Byron, *Inhaling medicines: delivering drugs to the body through the lungs.* Nat Rev Drug Discov, 2007. **6**(1): p. 67-74.

108. O'Riordan, T.G., L.B. Palmer, and G.C. Smaldone, *Aerosol deposition in mechanically ventilated patients. Optimizing nebulizer delivery.* Am J Respir Crit Care Med, 1994. **149**(1): p. 214-9.

109. Newman, S.P., *How well do in vitro particle size measurements predict drug delivery in vivo?* J Aerosol Med, 1998. **11 Suppl 1**: p. S97-104.

110. Newman, S.P. and H.K. Chan, *In vitro/in vivo comparisons in pulmonary drug delivery.* J Aerosol Med Pulm Drug Deliv, 2008. **21**(1): p. 77-84.

111. Taylor, K.M., *Pulmonary drug delivery*, in *Aulton's pharmaceutics: the design and manufacture of medicines*

M.E. Aulton, Editor. 2007, Elsevier Health Sciences: Philadelphia. p. 539-554.

112. Vecellio, L., *The mesh nebuliser: a recent technical innovation for aerosol delivery.* Breathe, 2006. **2**(3): p. 253-260.

113. Zainudin, B.M., et al., *Comparison of bronchodilator responses and deposition patterns of salbutamol inhaled from a pressurised metered dose inhaler, as a dry powder, and as a nebulised solution.* Thorax, 1990. **45**(6): p. 469-73.

114. Smith, E.C., J. Denyer, and A.H. Kendrick, *Comparison of twenty three nebulizer/compressor combinations for domiciliary use.* Eur Respir J, 1995. **8**(7): p. 1214-21.

115. Ari, A., et al., *A guide to Aerosol Delivery Devices for Respiratory Therapists*. 2nd ed. 2009, Dallas, Texas: American Association for Respiratory Care. 59.

116. Dolovich, M.B. and R. Dhand, *Aerosol drug delivery: developments in device design and clinical use.* Lancet, 2011. **377**(9770): p. 1032-45.

117. Rau, J.L., *Design principles of liquid nebulization devices currently in use.* Respir Care, 2002. **47**(11): p. 1257-75; discussion 1275-8.

118. Fok, T.F., et al., *Pulmonary deposition of salbutamol aerosol delivered by metered dose inhaler, jet nebulizer, and ultrasonic nebulizer in mechanically ventilated rabbits.* Pediatr Res, 1997. **42**(5): p. 721-7.

119. O'Doherty, M.J., et al., *Delivery of a nebulized aerosol to a lung model during mechanical ventilation. Effect of ventilator settings and nebulizer type, position, and volume of fill.* Am Rev Respir Dis, 1992. **146**(2): p. 383-8.

120. Harvey, C.J., et al., *Comparison of jet and ultrasonic nebulizer pulmonary aerosol deposition during mechanical ventilation.* Eur Respir J, 1997. **10**(4): p. 905-9.

121. Dhand, R., *Nebulizers that use a vibrating mesh or plate with multiple apertures to generate aerosol.* Respir Care, 2002. **47**(12): p. 1406-16; discussion 1416-8.

122. Waldrep, J.C. and R. Dhand, *Advanced nebulizer designs employing vibrating mesh/aperture plate technologies for aerosol generation.* Curr Drug Deliv, 2008. **5**(2): p. 114-9.

123. Rau, J.L., *The inhalation of drugs: advantages and problems.* Respiratory Care, 2005. **50**(3): p. 367-82.

124. Dhand, R., *New frontiers in aerosol delivery during mechanical ventilation.* Respir Care, 2004. **49**(6): p. 666-77.

125. Dubus, J.C., et al., *Aerosol deposition in neonatal ventilation.* Pediatric Research, 2005. **58**(1): p. 10-4.

126. Dhand, R., *Aerosol delivery during mechanical ventilation: from basic techniques to new devices.* J Aerosol Med Pulm Drug Deliv, 2008. **21**(1): p. 45-60.

127. Ari, A., et al., *Influence of nebulizer type, position, and bias flow on aerosol drug delivery in simulated pediatric and adult lung models during mechanical ventilation.* Respir Care, 2010. **55**(7): p. 845-51.

128. Pedersen, K.M., et al., *Factors influencing the in vitro deposition of tobramycin aerosol: a comparison of an ultrasonic nebulizer and a high-frequency vibrating mesh nebulizer.* J Aerosol Med, 2006. **19**(2): p. 175-83.

129. Ari, A., H. Areabi, and J.B. Fink, *Evaluation of aerosol generator devices at 3 locations in humidified and non-humidified circuits during adult mechanical ventilation.* Respir Care, 2010. **55**(7): p. 837-44.

130. Vecellio, L., et al., *Deposition of aerosols delivered by nasal route with jet and mesh nebulizers.* Int J Pharm, 2011. **407**(1-2): p. 87-94.

131. Weibel, E.R., *Morphometry of the human lung*. 1963, New York: Academic Press.

132. Patton, J.S., *Mechanisms of macromolecule absorption by the lungs.* Advanced Drug Delivery Reviews, 1996. **19**(1): p. 3-36.

133. Hogg, J.C., *Response of the lung to inhaled particles.* Medical Journal of Australia, 1985. **142**(13): p. 675-8.

134. Ward, H.E. and T.E. Nicholas, *Alveolar type I and type II cells.* Australian & New Zealand Journal of Medicine, 1984. **14**(5 Suppl 3): p. 731-4.

135. Gorin, A.B. and P.A. Stewart, *Differential permeability of endothelial and epithelial barriers to albumin flux.* J Appl Physiol Respir Environ Exerc Physiol, 1979. **47**(6): p. 1315-24.

136. Wangensteen, O.D., et al., *Tracheal epithelial permeability to nonelectrolytes: species differences.* J Appl Physiol (1985), 1993. **75**(2): p. 1009-18.

137. Byron, P.R. and E.M. Phillips, *Absorption, clearance and dissolution in the lung.* Respiratory Drug Delivery, 1999. **I**: p. 107-141.

138. Byron, P.R. and J.S. Patton, *Drug delivery via the respiratory tract.* J Aerosol Med, 1994. **7**(1): p. 49-75.

139. Effros, R.M. and G.R. Mason, *Measurements of pulmonary epithelial permeability in vivo.* Am Rev Respir Dis, 1983. **127**(5 Pt 2): p. S59-65.

140. Enna, S.J. and L.S. Schanker, *Absorption of drugs from the rat lung.* American Journal of Physiology, 1972. **223**(5): p. 1227-31.

141. Byron, P.R., *Determinants of drug and polypeptide bioavailability from aerosols delivered to the lung.* Advanced Drug Delivery Reviews, 1990. **5**(1–2): p. 107-132.

142. Patton, J.S., C.S. Fishburn, and J.G. Weers, *The lungs as a portal of entry for systemic drug delivery.* Proc Am Thorac Soc, 2004. **1**(4): p. 338-44.

143. Avram, M.J., et al., *Recirculatory pharmacokinetic model of the uptake, distribution, and bioavailability of prochlorperazine administered as a thermally generated aerosol in a single breath to dogs.* Drug Metab Dispos, 2007. **35**(2): p. 262-7.

144. Dershwitz, M., et al., *Pharmacokinetics and pharmacodynamics of inhaled versus intravenous morphine in healthy volunteers.* Anesthesiology, 2000. **93**(3): p. 619-28.

145. Stone, K.C., et al., *Allometric relationships of cell numbers and size in the mammalian lung.* Am J Respir Cell Mol Biol, 1992. **6**(2): p. 235-43.

146. Keith, I.M., et al., *Immunological identification and effects of 3-methylcholanthrene and phenobarbital on rat pulmonary cytochrome P-450.* Cancer Res, 1987. **47**(7): p. 1878-82.

147. Ji, C.M., et al., *Pulmonary cytochrome P-450 monooxygenase system and Clara cell differentiation in rats.* Am J Physiol, 1995. **269**(3 Pt 1): p. L394-402.

148. Tronde, A., et al., *Pulmonary absorption rate and bioavailability of drugs in vivo in rats: structure-absorption relationships and physicochemical profiling of inhaled drugs.* J Pharm Sci, 2003. **92**(6): p. 1216-33.

149. Brown, R.A., Jr. and L.S. Schanker, *Absorption of aerosolized drugs from the rat lung.* Drug Metab Dispos, 1983. **11**(4): p. 355-60.

150. Schanker, L.S., E.W. Mitchell, and R.A. Brown, Jr., *Species comparison of drug absorption from the lung after aerosol inhalation or intratracheal injection.* Drug Metab Dispos, 1986. **14**(1): p. 79-88.

151. Holford, N.H. and L.B. Sheiner, *Kinetics of pharmacologic response.* Pharmacol Ther, 1982. **16**(2): p. 143-66.

152. Food and Drug Administration, *Guidance for Industry: Clinical Pharmacology Data to Support a Demonstration of Biosimilarity to a Reference Product*, U.S.D.o.H.a.H. Services, Editor. 2014.

153. Prentice, R.L., *Surrogate endpoints in clinical trials: definition and operational criteria.* Stat Med, 1989. **8**(4): p. 431-40.

154. Lewis, J.A., *Statistical principles for clinical trials(ICH E 9): an introductory note on an international guideline.* Statistics in medicine, 1999. **18**(15): p. 1903-1942.

155. Haddad, F., et al., *Septal curvature is marker of hemodynamic, anatomical, and electromechanical ventricular interdependence in patients with pulmonary arterial hypertension.* Echocardiography, 2014. **31**(6): p. 699-707.

156. Laflamme, M., et al., *Preliminary experience with combined inhaled milrinone and prostacyclin in cardiac surgical patients with pulmonary hypertension.* J Cardiothorac Vasc Anesth, 2015. **29**(1): p. 38-45.

157. Mehrotra, N., et al., *The role of pharmacokinetics and pharmacodynamics in phosphodiesterase-5 inhibitor therapy.* Int J Impot Res, 2007. **19**(3): p. 253-64.

158. Gabrielsson, J. and D. Weiner, *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications*. 4 ed. 2006, Stockholm, Sweden: Swedish Pharmaceutical Press.

159. Wong, D., W.A. Colburn, and M. Gibaldi, *Fitting concentration--time data to biexponential equations.* J Pharmacokinet Biopharm, 1979. **7**(1): p. 97-100.

160. Karlsson, M.O. and R.M. Savic, *Diagnosing model diagnostics.* Clinical Pharmacology & Therapeutics. **82**(1): p. 17-20.

161. Ludden, T.M., S.L. Beal, and L.B. Sheiner, *Comparison of the Akaike Information Criterion, the Schwarz criterion and the F test as guides to model selection.* J Pharmacokinet Biopharm, 1994. **22**(5): p. 431-45.

162. Takimoto, C.H. and C.M. Ng, *Pharmacokinetics and Pharmacodynamics*, in *DeVita, Hellman, and Rosenberg's Cancer: Principles and Practice of Oncology*, V.T.J. DeVita, T.S. Lawrence, and S.A. Rosenberg, Editors. 2008, Wolters Kluwer/Lippincott Williams & Wilkins: Philadelphia. p. 392-400.

163. Kwon, Y., *Handbook of essential pharmacokinetics, pharmacodynamics and drug metabolism for industrial scientists*. 2001: Springer Science & Business Media.

164. Krzyzanski, W. and W.J. Jusko, *Integrated functions for four basic models of indirect pharmacodynamic response.* J Pharm Sci, 1998. **87**(1): p. 67-72.

165. Gavra, P., et al., *A specific and sensitive HPLC-MS/MS micromethod for milrinone plasma levels determination after inhalation in cardiac patients.* Ther Drug Monit, 2014. **36**(5): p. 663-8.

166. Ari, A. and J.B. Fink, *Factors affecting bronchodilator delivery in mechanically ventilated adults.* Nurs Crit Care, 2010. **15**(4): p. 192-203.

167. Gavra, P., et al., *High-performance liquid chromatography assay using ultraviolet detection for urinary quantification of milrinone concentrations in cardiac surgery patients undergoing cardiopulmonary bypass.* Biomed Chromatogr, 2014. **28**(8): p. 1084-9.

168. Miller, D.D., et al., *Aerosol delivery and modern mechanical ventilation: in vitro/in vivo evaluation.* Am J Respir Crit Care Med, 2003. **168**(10): p. 1205-9.

169. Fink, J.B., et al., *Aerosol delivery from a metered-dose inhaler during mechanical ventilation. An in vitro model.* Am J Respir Crit Care Med, 1996. **154**(2 Pt 1): p. 382-7.

170. Manthous, C.A., et al., *Metered-dose inhaler versus nebulized albuterol in mechanically ventilated patients.* Am Rev Respir Dis, 1993. **148**(6 Pt 1): p. 1567-70.

171. Manthous, C.A. and J.B. Hall, *Administration of therapeutic aerosols to mechanically ventilated patients.* Chest, 1994. **106**(2): p. 560-71.

172. Tuxen, D.V., *Permissive hypercapnic ventilation.* Am J Respir Crit Care Med, 1994. **150**(3): p. 870-4.

173. Hess, D.R., C. Dillman, and R.M. Kacmarek, *In vitro evaluation of aerosol bronchodilator delivery during mechanical ventilation: pressure-control vs. volume control ventilation.* Intensive Care Med, 2003. **29**(7): p. 1145-50.

174. Vecellio, L., et al., *In vitro study and semiempirical model for aerosol delivery control during mechanical ventilation.* Intensive Care Med, 2005. **31**(6): p. 871-6.

175. Holley, F.O., K.V. Ponganis, and D.R. Stanski, *Effect of cardiopulmonary bypass on the pharmacokinetics of drugs.* Clin Pharmacokinet, 1982. **7**(3): p. 234-51.

176. Buylaert, W.A., et al., *Cardiopulmonary bypass and the pharmacokinetics of drugs. An update.* Clin Pharmacokinet, 1989. **17**(1): p. 10-26.

177. Rosen, D.A. and K.R. Rosen, *Elimination of drugs and toxins during cardiopulmonary bypass.* J Cardiothorac Vasc Anesth, 1997. **11**(3): p. 337-40.

178. Mets, B., *The pharmacokinetics of anesthetic drugs and adjuvants during cardiopulmonary bypass.* Acta Anaesthesiol Scand, 2000. **44**(3): p. 261-73.

179. Morrell, D.F. and G.G. Harrison, *Lignocaine kinetics during cardiopulmonary bypass. Optimum dosage and the effects of haemodilution.* Br J Anaesth, 1983. **55**(12): p. 1173-7.

180. Plachetka, J.R., N.W. Salomon, and J.G. Copeland, *Plasma propranolol before, during, and after cardiopulmonary bypass.* Clin Pharmacol Ther, 1981. **30**(6): p. 745-51.

181. Moore, R.A., et al., *Effect of hypothermic cardiopulmonary bypass on nitroprusside metabolism.* Clin Pharmacol Ther, 1985. **37**(6): p. 680-3.

182. Koren, G., et al., *The influence of hypothermia on the disposition of fentanyl--human and animal studies.* Eur J Clin Pharmacol, 1987. **32**(4): p. 373-6.

183. Mathie, R.T., et al., *Hepatic blood flow during cardiopulmonary bypass operations: the effect of temperature and pulsatility.* J Thorac Cardiovasc Surg, 1997. **114**(2): p. 292-3.

184. Stanley, T.H., *Arterial pressure and deltoid muscle gas tensions during cardiopulmonary bypass in man.* Can Anaesth Soc J, 1978. **25**(4): p. 286-90.

185. Roth, R.A. and D.A. Wiersma, *Role of the lung in total body clearance of circulating drugs.* Clin Pharmacokinet, 1979. **4**(5): p. 355-67.

186. Bentley, J.B., T.J. Conahan, 3rd, and R.C. Cork, *Fentanyl sequestration in lungs during cardiopulmonary bypass.* Clin Pharmacol Ther, 1983. **34**(5): p. 703-6.

187. Hynynen, M., E. Hammaren, and P.H. Rosenberg, *Propofol sequestration within the extracorporeal circuit.* Can J Anaesth, 1994. **41**(7): p. 583-8.

188. Williams, G.D., et al., *Amrinone loading during cardiopulmonary bypass in neonates, infants, and children.* J Cardiothorac Vasc Anesth, 1995. **9**(3): p. 278-82.

189. Ramamoorthy, C., et al., *Pharmacokinetics and side effects of milrinone in infants and children after open heart surgery.* Anesth Analg, 1998. **86**(2): p. 283-9.

190. Bailey, J.M., et al., *The pharmacokinetics of milrinone in pediatric patients after cardiac surgery.* Anesthesiology, 1999. **90**(4): p. 1012-8.

191. Potts, A.L., et al., *Dexmedetomidine hemodynamics in children after cardiac surgery.* Paediatr Anaesth, 2010. **20**(5): p. 425-33.

192. El Sanharawi, M. and F. Naudet, *Comprendre la régression logistique.* Journal Français d'Ophtalmologie, 2013. **36**(8): p. 710-715.

# ANNEXE I. Manuscrit n°1 (chapitre de livre): A pathophysiological approach to understanding pulmonary hypertension in cardiac surgery

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# 1. Introduction

Pulmonary hypertension (PH) is associated with increased morbidity and mortality and is an important prognostic factor in cardiac surgery. As the average age and associated co-morbidities of cardiac surgical patients increase, the prevalence of PH is likely to rise. In this chapter, we will define PH, classify it on the basis of pathophysiological etiology, and suggest treatment therapies according to this classification. The importance of PH in cardiac surgery, its relationship to right ventricular dysfunction and preventive therapies will also be discussed. When applicable, we will draw from our clinical experience with PH to suggest strategies for the prevention of possible complications.

# 2. Definition of pulmonary hypertension

## 2.1. Hemodynamic parameters used in clinical settings

There are several hemodynamic parameters used in defining PH (Table **1**) (Gomez & Palazzo, 1998). These definitions have been used in various studies.

## 2.2. Diagnosis in awake and anesthetized patients

Pulmonary hypertension is usually diagnosed prior to cardiac surgery in awake patients. The diagnosis is obtained either directly by cardiac catheterization or indirectly by using Doppler signals from transesophageal echocardiography (TEE) and using Bernoulli's equation. In the presence of tricuspid regurgitation, the simplified Bernoulli's equation gives an estimation of the pressure gradient across the tricuspid valve (Fig. **1**) (Denault et al., 2010a). This pressure gradient is equal to the difference in systolic pressure between the right ventricle (RV) and the right atrium. Therefore, with the measurement of right atrial pressure (Pra), the estimation of systolic right ventricular pressure (Prv) is possible. In the absence of right ventricular outflow tract obstruction (RVOTO) and pulmonic valve stenosis, systolic Prv represents a reliable estimation of the systolic pulmonary artery pressure (sPAP).

**Table 1**. Definitions of Pulmonary Hypertension Used in Clinical Settings

| **Hemodynamic parameter** | **Normal value** | **Abnormal value** |
|---|---|---|
| Systolic pulmonary artery pressure (sPAP) | 15-30 mmHg | >30 or ≥40 mmHg |
| Mean pulmonary artery pressure (mPAP) | 9-16 mmHg | Moderate >18 mmHg<br>Significant >25 mmHg<br>Exercise-induced >30 mmHg |
| Pulmonary vascular resistance (PVR) = (mPAP – PAOP) X 80/CO | 60-120 dyn·sec·cm$^{-5}$ | Mild >125 dyn·sec·cm$^{-5}$<br>Moderate >200-300 dyn·sec·cm$^{-5}$<br>Severe >600 dyn·sec·cm$^{-5}$ |
| Indexed pulmonary vascular resistance (PVRI) = (mPAP – PAOP) X 80/CI | 250-340 dyn·sec·cm$^{-5}$·m$^{-2}$ | >340 dyn·sec·cm$^{-5}$·m$^{-2}$ |
| Pulmonary to systemic vascular resistance index (PVRI/SVRI) X 100% | ≤10% | >10% |
| Transpulmonary gradient (mPAP – PAOP) | ≤14 mmHg | >14 mmHg |
| Mean pulmonary to systemic pressure ratio (mPAP/mAP) X 100% | <25% | Moderate 33-50%<br>Severe >50% |
| Mean systemic to pulmonary pressure ratio (mAP/mPAP) | ≥4 | <4 |

CI: cardiac index; CO: cardiac output; mAP: mean arterial pressure; PAOP: pulmonary artery occlusion pressure; SVRI: indexed systemic vascular resistance. Adapted from Gomez *et al*. (Gomez & Palazzo, 1998).

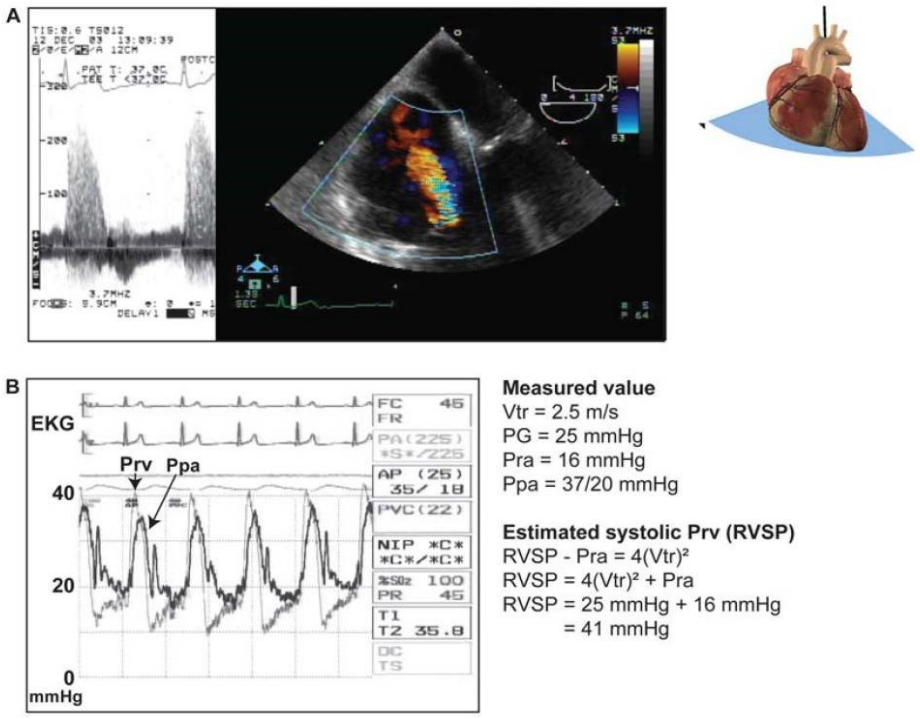


**Figure 1**. (**A**) Estimation of right ventricular systolic pressure (systolic Prv or RVSP) using the pressure gradient (PG) obtained from tricuspid regurgitation (TR) and right atrial pressure (Pra). (**B**) Note that the RVSP is higher than the systolic pulmonary artery pressure (Ppa) due to a small gradient across the pulmonic valve. (EKG: electrocardiogram; V: velocity). With permission from Denault *et al*. (Denault et al., 2010a).

## 2.3. Comparison of absolute and relative values in the assessment of pulmonary hypertension

Following the induction of general anesthesia, a reduction in both the systemic and the pulmonary artery pressures is observed. Consequently, using absolute values of sPAP in defining PH would underestimate its severity. To address this issue, Robitaille *et al*. studied 1557 patients undergoing cardiac surgery (Robitaille et al., 2006). In the 32 patients with preoperative PH, induction of general anesthesia resulted in a significant reduction in mean arterial pressure (mAP) and mean pulmonary artery pressure (mPAP) but the ratio of mAP/mPAP remained stable (Fig. **2**). The normal value for this ratio is >4, and lower values can be used to quantify the severity of PH.

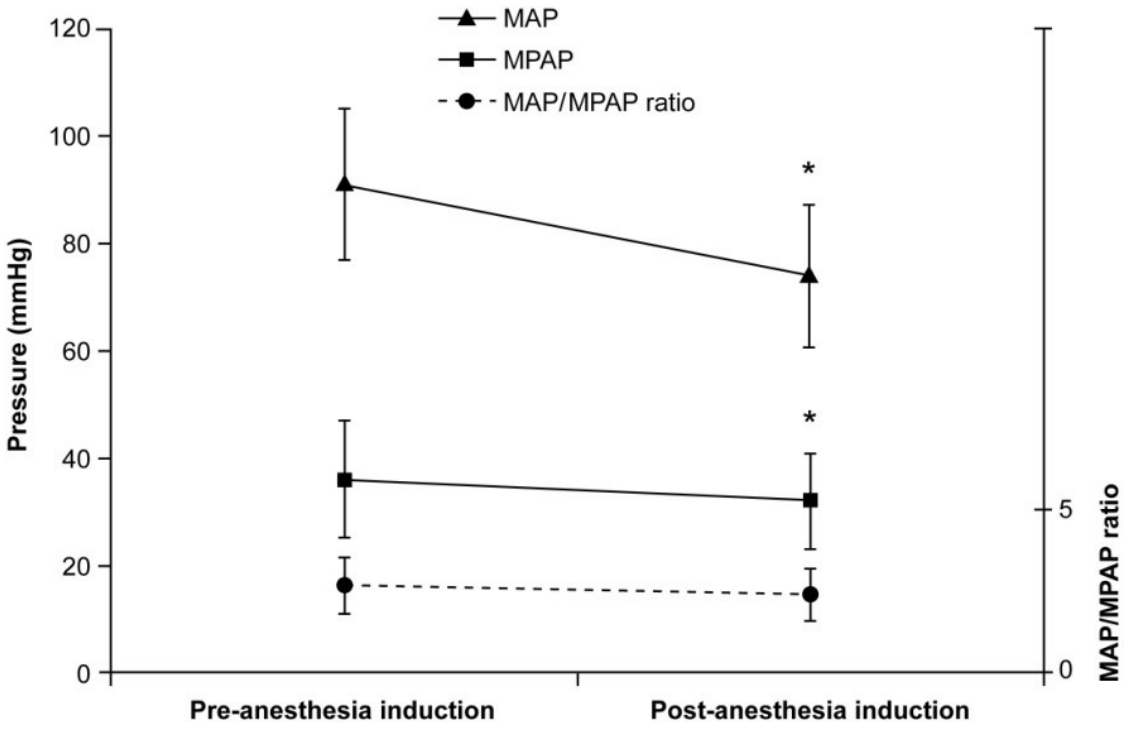


**Figure 2**. Changes in mean arterial pressure (mAP), mean pulmonary artery pressure (mPAP), and the mAP/mPAP ratio after the induction of anesthesia in 32 patients with preoperative pulmonary hypertension. No significant change in the mAP/mPAP ratio was observed (*p <0.05). (Robitaille et al., 2006)

The relevance of the mAP/mPAP ratio was demonstrated after comparing its ability to estimate the probability of postoperative complications with the ability of other normally used hemodynamic parameters for this purpose (listed in Table **1**). Values of the ratio obtained after induction of general anesthesia but before cardiopulmonary bypass (CPB) in 1439 patients undergoing cardiac surgery showed similar trend when compared to other hemodynamic parameters (Fig. **3**). Furthermore, the ratio turned out to be the best predictor of perioperative complications, defined as death, need for intra-aortic balloon pump, cardiac arrest, or use of vasoactive support for more than 24 hours.

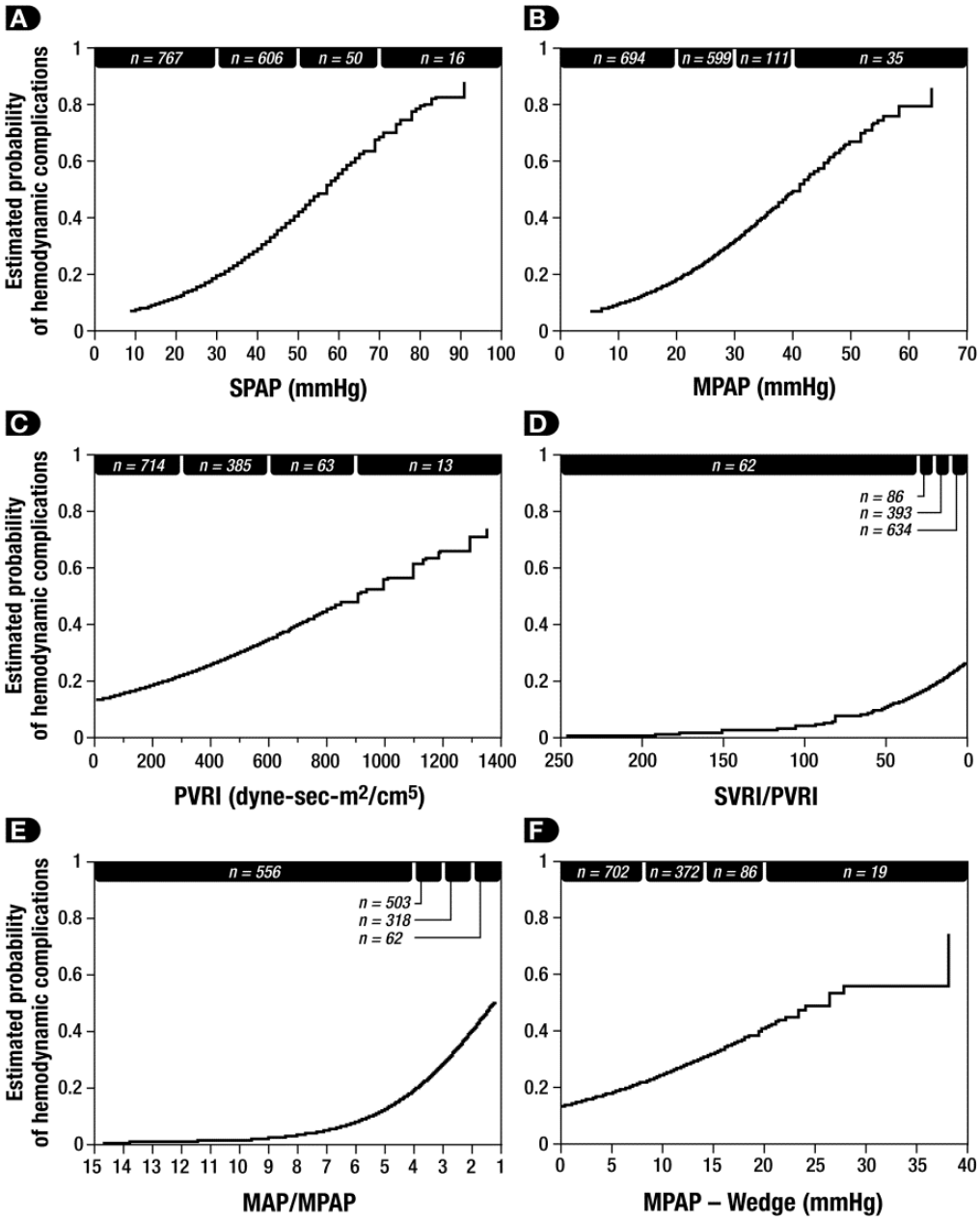


**Figure 3**. Relationship between the estimated probability of hemodynamic complications and variables used in the evaluation of pulmonary hypertension: (**A**) systolic pulmonary artery pressure (sPAP), (**B**) mean pulmonary artery pressure (mPAP), (**C**) indexed pulmonary vascular resistance (PVRI), (**D**) systemic to pulmonary vascular resistance index ratio (SVRI/PVRI), (**E**) mean systemic to pulmonary pressure ratio (mAP/mPAP), and (**F**) transpulmonary gradient defined as mPAP-Wedge or pulmonary artery occlusion pressure (PCWP or PAOP). For easier comparison, the scale of the x axis of the SVRI/PVRI and the mAP/mPAP are inverted. (n=number of patients). (Robitaille et al., 2006)

An abnormal mAP/mPAP ratio was also recognized to be significantly correlated with abnormal systolic and/or diastolic cardiac function (Fig. **4**) (Robitaille et al., 2006). The use of relative instead of absolute values to estimate PH is currently used in congenital cardiology (Therrien et al., 2001a; Therrien et al., 2001b).

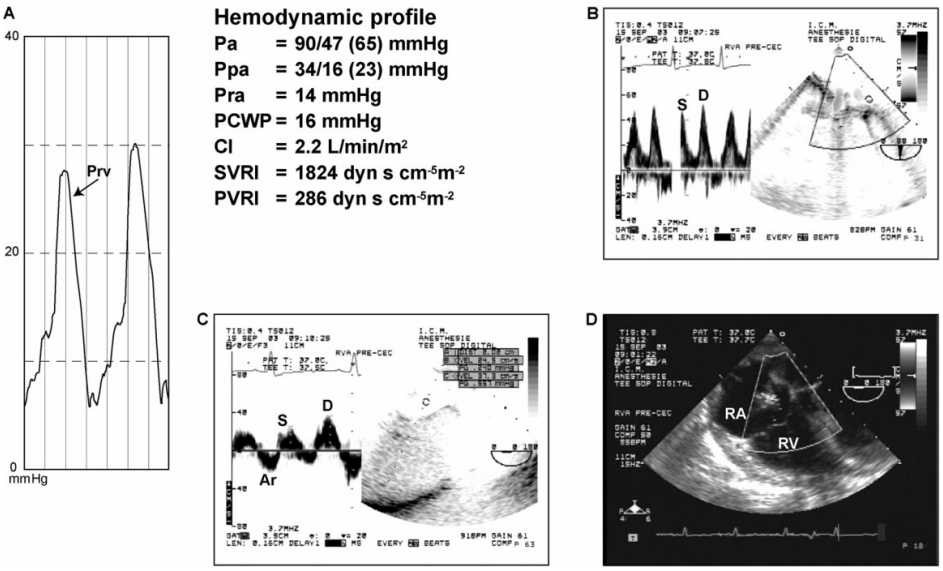


**Figure 4**. Hemodynamic and transesophageal echocardiographic evaluation of a 46-year-old woman scheduled for aortic valve surgery. Despite a normal pulmonary artery pressure (Ppa) of 34/16 mmHg and pulmonary vascular resistance index (PVRI) at 286 dyn·s·cm$^{-5}$·m$^{-2}$, this patient had an abnormal right ventricular diastolic filling pressure waveform characterized by a rapid upstroke (**A**) and reduced systolic (S) to diastolic (D) pulmonary (**B**) and hepatic (**C**) venous flows consistent with left and right ventricular diastolic dysfunction. In addition, a dilated right atrium and ventricle were present without significant tricuspid regurgitation in a mid-esophageal right ventricular view (**D**). The mean systemic to pulmonary pressure ratio (mAP/mPAP) ratio was 65/23 or 2.8. (CI: cardiac index; Pa: arterial pressure; PCWP: pulmonary capillary wedge pressure; Pra: right atrial pressure; Prv: right ventricular pressure; RA: right atrium; RV: right ventricle; SVRI: systemic vascular resistance index). (Robitaille et al., 2006)

In summary, the evaluation and diagnostic of PH in cardiac surgical patients must be done using specific criteria. In awake patients, the absolute values can be used since they correlate well with outcomes. However, in patients under general anesthesia, the ratio of mAP/mPAP allows to screen for PH when systolic blood pressures are lower due to the anesthetic agents.

# 3. Classification of pulmonary hypertension based on pathophysiology and etiology

The 2008 World Symposium on PH endorsed by The World Health Organization (WHO) proposed a classification system divided into 5 groups: 1) Pulmonary arterial hypertension, 2) PH owing to left heart disease, 3) PH owing to lung diseases and/or hypoxia, 4) Chronic thromboembolic PH, and 5) PH with unclear or multifactorial etiologies (Simonneau et al., 2009). In cardiac surgery, PH is more frequently classified as pre-capillary, capillary or post-capillary, depending on the site where the underlying cause of PH is found. In this context, PH during cardiac surgery is typically post-capillary since the cause is mainly of left ventricular (LV) origin, past the pulmonary capillary bed. To confirm this diagnosis, pulmonary artery catheterization can be used to demonstrate an equal value for diastolic pulmonary artery pressure (dPAP) and pulmonary artery occlusion pressure (PAOP). When the cause for PH is at the pre-capillary or capillary level, in absence of tachycardia, dPAP is significantly higher than PAOP (Gomez & Palazzo, 1998).

The causes underlying PH in cardiac surgery can be complex and may result from several mechanisms acting alone or in combination (Fig. **5**). These mechanisms may exist before the operation or appear during or after the procedure. Exacerbation of PH may happen at any time during cardiac surgery, before, during or after CPB. Indeed, patients are at risk of LV failure at all times, especially after CPB when the reperfusion of the ischemic lungs can cause pulmonary reperfusion syndrome. Finally, PH can persist postoperatively secondary to a patient-prosthesis-mismatch (PPM) after mitral or aortic valve replacement. The treatment of PH is based on the identification of its etiology, whence the importance of distinguishing between the different pathophysiologies.

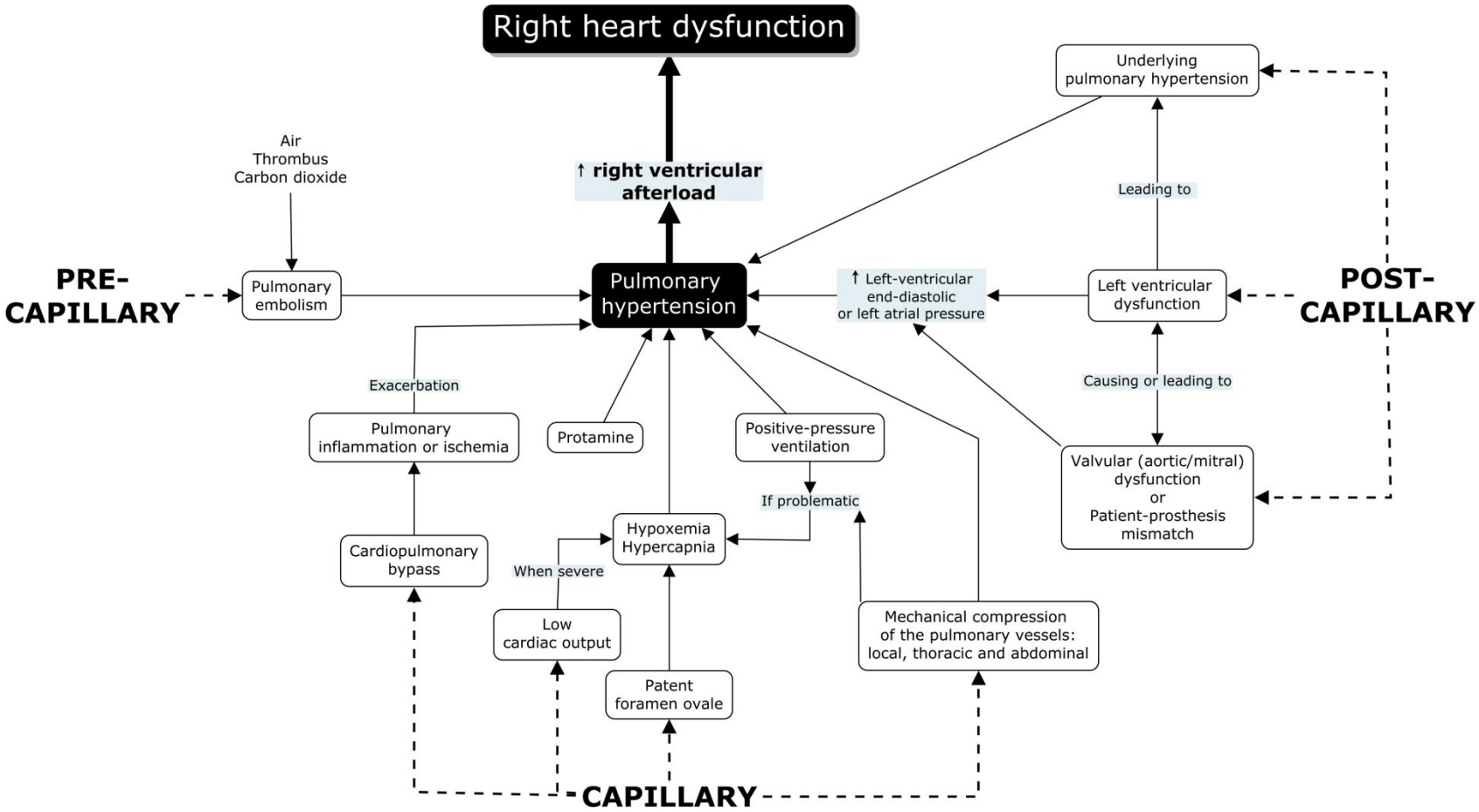


**Figure 5**. Major mechanisms of pulmonary hypertension in cardiac surgery. Other mechanisms may be operating at several levels: for instance, hypoxia (capillary) may lead to pulmonary hypertension, right ventricular systolic failure and, through interventricular interaction, left ventricular diastolic function (post-capillary).

## 3.1. Review of the factors involved

The most important causes of PH in cardiac surgery, illustrated in Fig. **5**, are classified according to their originating anatomical site: pre-capillary, capillary and post-capillary.

### 3.1.1. Pre-capillary

**Pulmonary embolism**

Pulmonary embolism is an example of a pre-capillary PH. It may occur before, during or after CPB leading to the development or the exacerbation of PH. Thrombus, air and even carbon dioxide (Martineau et al., 2003) can cause pulmonary embolism. Pulmonary embolisms are rare in the immediate cardiac postoperative period. However, patients at risk include patients with predisposing factors to PH and patients with chronic thromboembolic pulmonary hypertension (CTEPH) (Fig. **6**). The incidence of CTEPH is uncertain, but it represents a frequent cause of PH occurring in up to 4% of patients after an acute pulmonary embolism (Pengo et al., 2004; Tapson & Humbert, 2006).

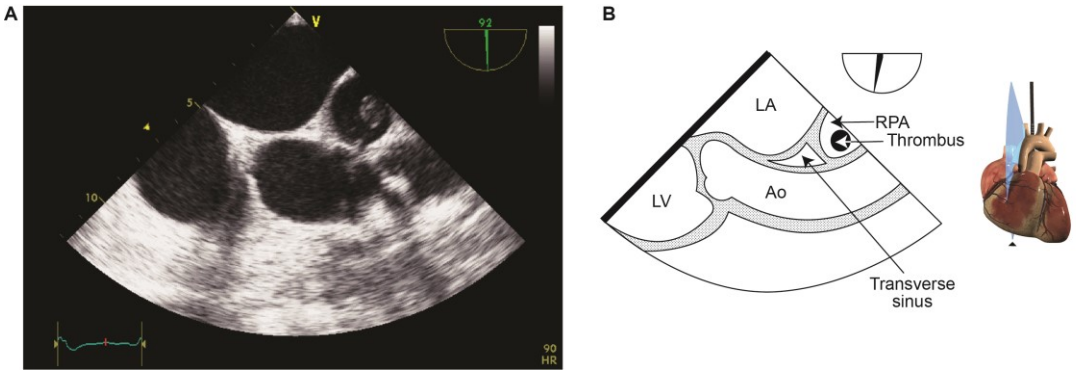


**Figure 6**. Chronic pulmonary embolism. (**A**, **B**) Mid-esophageal ascending aorta (Ao) long-axis view in a 65-year-old woman with chronic pulmonary embolism shows the mobile clot adherent to the right pulmonary artery (RPA) wall. (LA: left atrium; LV: left ventricle). With permission from Denault *et al*. (Denault et al., 2010a).

### 3.1.2. Capillary

**Cardiopulmonary bypass**

Pulmonary damage during CPB is one of the important etiologies of PH in cardiac surgery. This is mainly due to the fact that the lungs are ischemic during CPB. The underlying mechanisms include 1) release of cytokines through endotoxin production

(Downing & Edmunds, Jr., 1992), 2) complement activation and 3) ischemia reperfusion injury (Wan et al., 1997; Asimakopoulos et al., 1999) which leads to the production of free radicals, endothelin and prostacyclin derivatives with nitric oxide inhibition (Wan et al., 1997). The resulting systemic inflammatory response, pulmonary reperfusion syndrome as well as the transfusion of blood products may all exacerbate PH (Fig. **7**) (Lesage et al., 1966; Kaul & Fields, 2000).

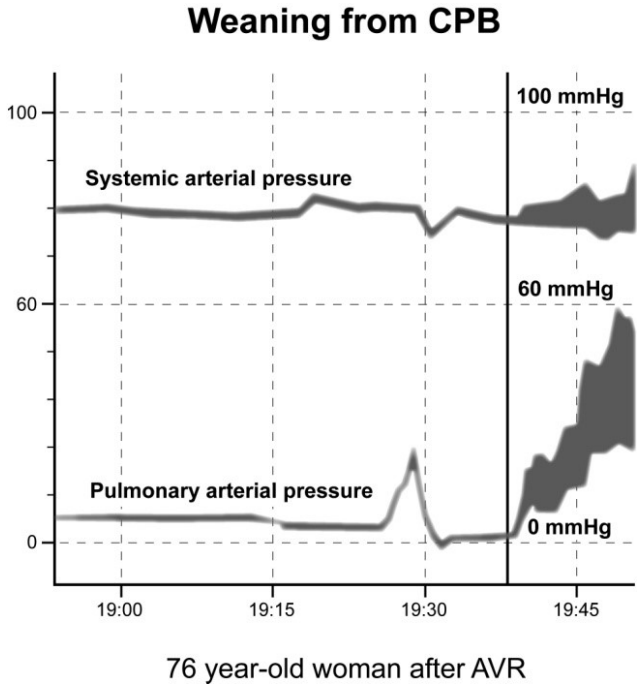


**Figure 7**. Unexpected pulmonary hypertension upon weaning from cardiopulmonary bypass (CPB) in a 76 year-old woman after aortic valve replacement (AVR). The CPB duration was 71 minutes. A significant increase in pulmonary arterial pressure in relation to the systemic arterial pressure was observed as the patient was weaned from CPB. No mechanical causes were found.

During CPB, blood is exposed to an artificial surface for oxygenation before it is sent back into the systemic circulation. This is associated with an inflammatory reaction secondary to endothelial activation, activation of the complement cascade, neutrophils, thrombin and platelets. Since the heart and lungs do not receive blood during CPB, cardioplegia solutions are used to preserve heart function, however, no specific protection is undertaken for the pulmonary circulation. In some patients, this may result in pulmonary reperfusion syndrome associated with postoperative endothelial

dysfunction and PH or in post-CPB respiratory distress syndrome. The latter phenomenon, similar to the respiratory distress syndrome in adults, is characterized by an increased capillary permeability leading to a reduction in oxygenation, increased alveolar-arterial gradient, decreased lung compliance, increased pulmonary vascular resistance (PVR), and exacerbation of preoperative PH. Activation of the endothelin system during CPB increases endothelin (ET-1) concentrations and correlates with CPB duration, severity of PH and post-CPB myocardial dysfunction. For this reason, CPB duration plays a major role in the incidence of mortality in cardiac surgery. Post-CPB PH can lead to RV dysfunction which, when severe, is fraught with a 44 to 86% mortality rate.

**Protamine**

The administration of protamine can induce catastrophic pulmonary vasoconstriction in up to 1.8% of patients (Ocal et al., 2005). Protamine is administered in CPB to neutralize the anti-clotting effects of heparin and has the capacity to activate the complement cascade. Thus, when given at the end of CPB, it can induce PH associated with adverse hemodynamic responses that range from minor perturbations to cardiovascular collapse, and may occur in three forms: systemic hypotension, anaphylactoid reaction and catastrophic PH (Viaro et al., 2002). The mechanism of protamine-induced PH is thought to be caused by an imbalance of vasoconstrictors and vasodilators leading to a reduction in nitric oxide release from the pulmonary vasculature (Viaro et al., 2002).

**Lung diseases and/or hypoxia**

In this category, the predominant cause of PH is alveolar hypoxia as a result of impaired control of breathing or lung disease.

Lung volumes exert a differential effect on the resistance of intra- and extra-alveolar vessels, which accounts for the unique U-shaped relationship between lung volume and PVR (Fig. **8**). At functional residual capacity (FRC), PVR is minimal but increases at large or total lung capacity (TLC) and small lung volumes. Clinically, this may be observed when hyperinflation of the lungs greatly increases PVR (Fischer et al., 2003).

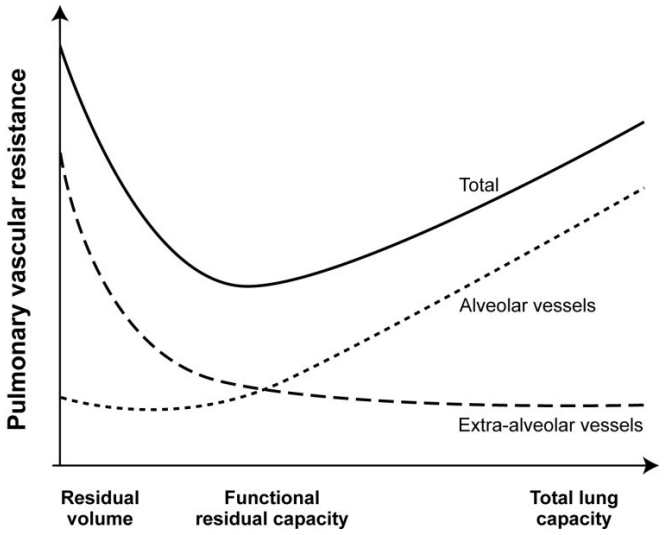


**Figure 8**. Relationship between lung volume and pulmonary vascular resistance (PVR). At functional residual capacity (FRC) PVR is minimal and increases at large or total lung capacity (TLC) and residual volume decreases at small lung volumes. The differential effect on intra and extra-alveolar vessels accounts for the U-shaped relationship of PVR and lung volume. Adapted from Fischer *et al*. (Fischer et al., 2003).

Changes in cardiac output (CO), airway pressure, and gravity may affect the pulmonary circulation. Therefore, patients with PH already have a restricted pulmonary circulation and increases in oxygen demand may further worsen PH and lead to right heart failure.

Application of high levels of positive end-expiratory pressure (PEEP) may narrow capillaries in well-ventilated lung areas (intra-alveolar) and divert blood flow to less-well ventilated or non-ventilated areas (extra-alveolar). Thus, intrapulmonary shunts may result in desaturation of mixed venous blood, potentially leading to hypoxia.

Hypoxia may also be caused by right-to-left intracardiac shunting through a patent foramen ovale (PFO) or a congenital heart defect. Pulmonary hypertension can lead to RV dysfunction causing increased pressure in the right atrium. In turn, the increase in Pra may result in opening of a PFO, present in 20-30% of the general population (Sukernik et al., 2001), increasing the severity of hypoxia. In contrast to systemic arteries, pulmonary vessels constrict with hypoxia (Euler-Liljestrand reflex) and dilate in

the presence of hyperoxia (Fischer et al., 2003), which explains the exacerbation of PH with hypoxia.

Hypercapnia can occur especially in the case of acute lung injury during or after the procedure. The increase in partial pressure of carbon dioxide ($PCO_2$) will cause vasoconstriction and therefore worsen PH.

Increases in CO distend open vessels and recruit previously closed vessels so that when the cross-sectional area of pulmonary circulation increases, PVR decreases.

Mechanical compression of pulmonary vessels is transmitted to the surrounding cardiac pressure and contributes to increase PAP. Hemothorax or tension pneumothorax may be responsible for an elevation in intrathoracic pressure.

In addition, gravity influences blood flow in the pulmonary circulation. Both regional blood flow and ventilation are greater in the dependent areas of the lung (intra-alveolar). Hence, the relationship between alveolar and hydrostatic pressure bears important clinical consequences.

Multiple molecular pathways are involved in the regulation of PVR, namely nitric oxide, prostacyclin, endothelin-1 and serotonin pathways (Humbert et al., 2004). Nitric oxide and prostacyclin are endogenous vasodilators produced in the pulmonary vascular endothelium. Endothelin-1 is an endogenous vasoconstrictor peptide secreted by the vascular endothelium and plays a role in pulmonary vasoconstriction and vascular smooth muscle proliferation (McLaughlin & McGoon, 2006). The neurotransmitter serotonin and the serotonin receptor transporter are also involved in the regulation of pulmonary vascular tone. Therefore, an imbalance in these pathways may result in vasoconstriction and vascular remodelling, potentially leading to progressive pulmonary vascular disease.

### 3.1.3. Post-capillary

**Left heart disease**

Left ventricular disease represents the most frequent cause of PH in cardiac surgery (Oudiz, 2007). Left-sided dysfunction includes three distinct etiologies: systolic dysfunction, diastolic dysfunction, and valvular heart disease (mitral and/or aortic). Pre-

or postoperative left-sided ventricular or valvular diseases may produce an increase in left atrial pressure, with passive backward transmission of the pressure leading to increased PAP. The elevation of PAP and PVR is due to either the increase of pulmonary artery vasomotor tone and/or pulmonary vascular remodeling (Delgado et al., 2005; Moraes et al., 2000).

**Patient-prosthesis mismatch**

Aortic PPM through a reduction in coronary reserve would also contribute to postoperative PH (Bakhtiary et al., 2007) and persistent post-operative valvular gradients (Fig. **9**).

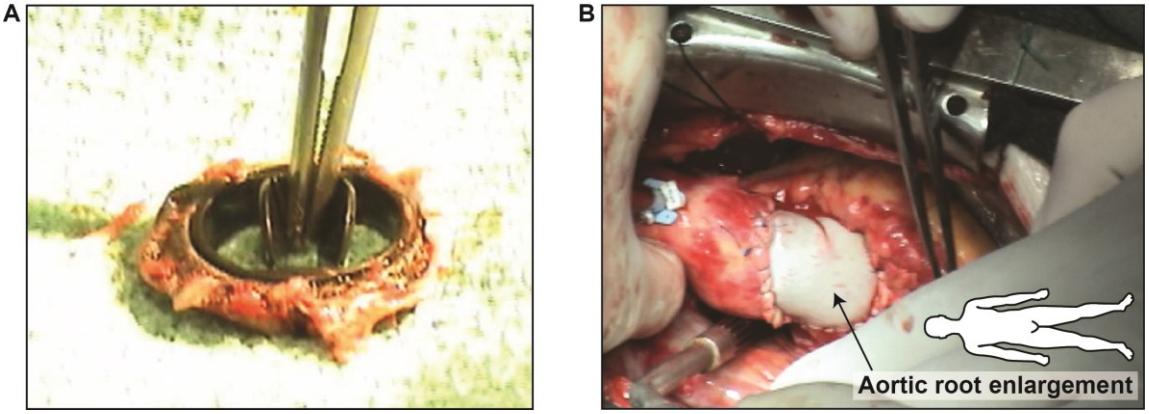


**Figure 9**. Patient-prosthesis aortic valve mismatch. A 71-year-old man with a body surface area of 1.89 $m^2$ was re-operated for symptoms of severe aortic valve stenosis. He had an AVR 4 years before with a Carbomedics 19 mm mechanical bileaflet prosthesis (non-indexed effective orifice area=1.06 $cm^2$). (**A**) The preoperative mean gradient was 41 mmHg although the intraoperative inspection of the prosthetic valve was completely normal. (**B**) Intraoperative view of an aortic root enlargement procedure in a 69-year-old patient with a reduced aortic diameter requiring AVR. Courtesy of Dr. Michel Carrier. With permission from Denault *et al*. (Denault et al., 2010a).

There is general agreement that the postoperative indexed effective orifice area (EOA) of the prosthesis being implanted should not be <0.85 to 0.90 $cm^2/m^2$. Mitral PPM was recently described as another cause of residual postoperative PH. Magne *et al*. (Magne et al., 2007) studied 929 patients who underwent mitral valve replacement (MVR), following them up to 15 years. Mitral valve PPM was defined according to the indexed

valve EOA as not clinically significant (EOA >1.2 $cm^2/m^2$), moderate (1.2 $cm^2/m^2$ ≥EOA >0.9 $cm^2/m^2$), and severe (EOA ≤0.9 $cm^2/m^2$). Prevalence of moderate and severe PPM was 69% and 9%, respectively. In addition, severe PPM was found to be associated with residual PH and a 3-fold increase in postoperative mortality after adjustment for other risk factors. This relevant new finding is currently absent from the majority of studies involving predictors of survival in mitral valve surgery.

# 4. Treatment of pulmonary hypertension in cardiac surgery based on pathophysiology and etiology

The approach to pharmacological and non-pharmacological treatment of PH will be directed towards the cause or the consequence of PH, as illustrated in Fig. **5**. Most often, treatment of the underlying mechanism causing PH requires non-pharmacological approaches, while pharmacological approaches will usually be the solution for the treatment of persisting PH and its consequence, RV failure.

## 4.1. Pharmacological and non-pharmacological approaches

Therapeutic management of PH has dramatically improved in the last years, offering both relief from symptoms and prolonged survival. However, there is still no cure for this disease. Moreover, in presence of PH, the choice of the appropriate therapy should rely on evidence-based medicine. By performing a Medline search using the keywords 'randomized controlled trial', 'humans', 'adults', 'pulmonary hypertension' and 'English', a total of 14 articles in cardiac surgery were retrieved. These publications were then classified according to their levels of evidence (Sackett, 1989; Moher et al., 2001) and summarized in Table **2**. Most of the studies reviewed were based on a small number of patients and had hemodynamic changes as their primary end-points. Various pharmacological agents were studied: inhaled prostacyclin $I_2$ ($iPGI_2$), inhaled nitric oxide (iNO), heparinase, protamine and intravenous vasodilators including prostaglandin $E_1$ ($PGE_1$), nitroglycerin (NTG), nitroprusside, milrinone, enoximone, dobutamine, oral sildenafil, beraprost and oxygen. Findings on pharmacological and non-pharmacological approaches for the treatment of PH in cardiac surgery will be discussed together in this section.

**Table 2**. Randomized Controlled Trial in the Treatment of Pulmonary Hypertension in Adult Cardiac Surgery

| Author | Country | Date | Agents used | Design | N | Inclusion criteria | Primary end-point | Efficacy | Level of evidence |
|---|---|---|---|---|---|---|---|---|---|
| Fernandes *et al*. | Brazil | 2011 | iNO *vs* oxygen | Single-center | 29 | MVR + PH after surgery | Hemodynamic | Yes | A1b |
| Kim *et al*. | Korea | 2010 | oral sildenafil + beraprost *vs* placebo | Single-center | 50 | PH before surgery | Hemodynamic | No | A1b |
| Khan *et al*. | USA | 2009 | $iPGI_2$ *vs* iNO | Crossover Single-center | 25 | PH, refractory hypoxemia, or RV dysfunction | Hemodynamic and oxygenation | Idem | A1b |
| Wang *et al*. | China | 2009 | Inhaled milrinone *vs* intravenous milrinone | Single-center | 48 | MVR + PH after surgery | Hemodynamic | Yes | A1b |
| Fattouch *et al*. | Italy | 2006 | $iPGI_2$ *vs* iNO vs intravenous vasodilators | Single-center | 58 | MVR + PH before the end of CPB | Hemodynamic | Yes | A1b |
| Ocal *et al*. | Turkey | 2005 | $iPGI_2$ *vs* NTG | Multicenter | 68 | CABG with protamine reaction after CPB | Hemodynamic | Yes | A1b |
| Stafford *et al*. | USA | 2005 | Heparinase *vs* protamine | Multicenter | 167 | CABG on + off pump after CPB | Bleeding | No | A1b |
| Fattouch *et al*. | Italy | 2005 | $iPGI_2$ *vs* iNO *vs* intravenous vasodilators | Single-center | 58 | MVR + PH in the intensive care unit | Hemodynamic | Yes | A1b |
| Hache *et al*. | Canada | 2003 | $iPGI_2$ *vs* placebo | Single-center | 20 | PH before CPB | Hemodynamic | Yes | A1b |
| Solina *et al*. | USA | 2001 | iNO *vs* milrinone | Single-center | 62 | PH after surgery | Hemodynamic | Yes | B |
| Feneck *et al*. | UK | 2001 | Milrinone *vs* dobutamine | Multicenter | 120 | CO <2 L/min/m² et PAOP >10 mmHg after cardiac surgery | Hemodynamic | Idem | A1b |
| Solina *et al*. | USA | 2000 | iNO *vs* milrinone | Single-center | 45 | PH after surgery | Hemodynamic | Yes | A1b |
| Schmid *et al*. | Switzerland | 1999 | iNO *vs* NTG *vs* $PGE_1$ | Crossover Single-center | 14 | PH after surgery | Hemodynamic | Idem | B |
| Hachenberg *et al*. | Germany | 1997 | Enoximone *vs* dobutamine+NTG | Single-center | 20 | PH in MVR before and after surgery | Hemodynamic | Idem | A1b |

CABG: coronary artery bypass graft; CO: cardiac output; CPB: cardiopulmonary bypass; iNO: inhaled nitric oxide; $iPGI_2$: inhaled prostacyclin; MVR: mitral valve replacement; NO: nitric oxide; NTG: nitroglycerin; PAOP: pulmonary artery occlusion pressure; $PGE_1$: prostaglandin $E_1$; $PGI_2$: prostacyclin; PH: pulmonary hypertension; RCT: randomized controlled trial; UK: United Kingdom; USA: United States of America.

### 4.1.1. Pre-capillary

**Pulmonary embolism**

Acute pulmonary embolism during cardiac surgery can lead to PH and, in some cases, evolve into CTEPH. Pulmonary thromboembolectomy, when surgically indicated, can help control PH and is currently the only curative treatment in patients with CTEPH (Jamieson & Nomura, 2000; Jamieson et al., 2003) (Fig. **6**). In case of CTEPH, evaluation of the feasibility of surgery mainly depends on the location of the obstruction (central vs. more distal pulmonary arteries) (Dartevelle et al., 2004). Patients who are not candidates for surgery may also benefit from PH-specific medical therapy, however, the use of these medications in CTEPH requires further evaluation in randomized controlled trials (Jais et al., 2008; Rubin et al., 2006; Suntharalingam et al., 2008).

The rationale for systemic anticoagulant therapy for chronic lung embolism in patients with PH may be justified by well-recognized risk factors for venous thromboembolism, such as heart failure, a sedentary lifestyle, and a thrombophilic predisposition (Bjornsson & Edwards, 1985). However, no data actually support anticoagulant therapy specifically in patients with PH. Warfarin has been evaluated in only two nonrandomized studies, one retrospective and the other prospective, involving a small number of patients (Fuster et al., 1984; Rich et al., 1992).

### 4.1.2. Capillary

**Cardiopulmonary bypass**

As discussed, CPB causes pulmonary damage during surgery through different mechanisms, potentially leading to PH but, more frequently, it contributes to the exacerbation of PH caused by other factors during the surgical procedure. In this context, patients can benefit from PH-specific medical therapy (Table **2**) and prophylactic treatments for PH use in cardiac surgery, which will be discussed later in this chapter.

In 62 patients with preoperative PH (PVR >125 dyn·sec·cm$^{-5}$ immediately before induction of anesthesia) Solina et *al*. (Solina et al., 2001) explored the dose-responsiveness of 10, 20, 30 and 40 ppm of iNO administered upon termination of CPB

in comparison to an intravenous bolus of 50 mg/kg of milrinone given 15 minutes before separation from CPB followed by a 0.5 mg/kg/min regimen administered in the operating room thereafter. Treatment with iNO was associated with significant reductions in PVR at all doses but no improved benefit was observed for doses higher than 10 ppm. No significant difference was observed between iNO and milrinone in terms of reduction in PVR and inotropic requirement.

The same team compared 20 and 40 ppm of iNO to the same dose of intravenous milrinone in 45 patients after cardiac surgery (Solina et al., 2000). Study drugs were administered upon termination of CPB for a 24h-period in the intensive care unit (ICU). The group receiving 20 ppm iNO had a significantly higher mAP while the group receiving 40 ppm had higher right ventricular ejection fraction (RVEF) on arrival in the ICU. The milrinone group required significantly more phenylephrine in the ICU with a trend towards higher heart rates.

In a crossover study, Schmid et *al*. (Schmid et al., 1999) compared iNO, $PGE_1$ and NTG in 14 adult patients with severe PH (mPAP >30 mmHg; PVR >300 dyn·sec·$cm^{-5}$) after cardiac surgery. The investigation was performed in the ICU within the first 24 h after the procedure. The generalization of results obtained from this study was limited, since it only included patients in stable postoperative circulatory conditions. However, in contrast to $PGE_1$ and NTG, iNO decreased PVR without exerting concomitant systemic vasodilatory effects. In addition, iNO did not affect the right coronary perfusion pressure and increased oxygen transport.

**Protamine**

The administration of protamine can be associated with severe PH followed by RV failure. This condition requires immediate treatment. In coronary artery bypass graft (CABG) patients (*n*=3800), Ocal *et al*. (Ocal et al., 2005), two therapeutic approaches were compared for the treatment of the protamine reaction observed in 68 of them (1.8%). One group received $iPGI_2$ and the other intravenous NTG in addition to standard vasoactive agents. The $iPGI_2$ group showed improved hemodynamics and only 14 patients (39%) had to return to CPB compared with all 30 patients (100%) in the NTG group. A trend towards a shorter length of stay in the ICU and reduced mortality was

observed in the $iPGI_2$ group, but the numbers were too small to achieve statistical significance.

In order to avoid protamine reaction, heparinase I, a heparin degrading enzyme, was compared to protamine in a multicenter randomized controlled trial (Stafford-Smith et al., 2005). The prevention of protamine-induced PH was also explored as a secondary end-point. Heparinase I was not associated with a reduction in bleeding or reduction in the need for intervention in the treatment of PH.

**Lung diseases and/or hypoxia**

Low CO during cardiac surgery may affect the pulmonary circulation, potentially leading to hypoxia and worsen PH and RV failure. Thus, an acute perioperative low-output state should be reversed whenever possible before clinical manifestation of chronic hypoperfusion and organ dysfunction.

Khan et *al*. (Khan et al., 2009) compared iNO to $iPGI_2$ in 25 heart and lung transplant recipients with PH, refractory hypoxia, or RV dysfunction. Patients were randomized to iNO (20 ppm) or $iPGI_2$ (20,000 ng/ml) as initial treatment in the operating room, followed by a crossover to the other agent after 6 hours. Both iNO and $iPGI_2$ reduced PAP and central venous pressure (CVP), and improved cardiac index (CI) and mixed venous oxygen saturation on initiation of therapy. At the 6-hour crossover trial, there were no significant differences between groups in the reduction of PAP and CVP, and the improvement of CI and mixed venous oxygen saturation on initiation of therapy. Neither iNO nor $iPGI_2$ affected the oxygenation index or systemic blood pressure.

In the case of chronic hypoxia, supplemental oxygen may be indicated to maintain arterial oxygen saturation at a level above 90 percent (Rubin & Rich, 1997).

In the presence of lung disease, improvement of symptoms of PH may be obtained using basic therapy for PH, for instance, therapy for chronic obstructive pulmonary disease (COPD) and corticosteroids for interstitial lung disease. Antibiotic therapy for pneumonia as well as elimination of ventilation/perfusion mismatch from and atelectasis can also help control PH.

Chest drainage is required in patients with elevated intrathoracic pressure resulting from accumulated air or blood. However, chest closure may be associated with hemodynamic instability in patients requiring long procedures associated with prolonged CPB due to myocardial edema. The solution to this "thoracic compartment syndrome" consists in leaving the chest temporarily opened in order to reduce surrounding pressures until edema recedes.

### 4.1.3. Post-capillary

**Left heart disease**

Left and right ventricular functions are interdependent. All LV function abnormalities induced by coronary artery disease, congestive heart failure, valvular heart disease, or systemic hypertension will influence RV function through ventricular interdependence mainly through an effect on the interventricular septum. Hence, a dilated LV and left atrium can shift the interatrial and interventricular septum and compress the right atrium and ventricule and reduce RV end-diastolic volume.

Fernandes *et al*. (Fernandes et al., 2011) compared iNO to oxygen in 29 patients with PH after MVR. Treatments were initiated for 48 hours immediately after surgery. After 24 and 48 hours, patients receiving iNO had a significantly greater increase in CI compared to patients receiving oxygen ($p < 0.0001$). Pulmonary vascular resistance was also more significantly reduced in patients receiving iNO versus oxygen ($p=0.005$) at 48 hours. Patients in the iNO group required less systemic vasoactive drugs and had a shorter ICU stay ($p=0.02$).

Kim *et al*. (Kim et al., 2010) compared the pulmonary vasodilation effect of combined preoperative oral sildenafil (50 mg) and beraprost (40 μg) (pulmonary vasodilators) to placebo in 50 patients scheduled for valvular heart surgery with PH (mPAP >30 mmHg). Medication was initiated 15 min before the induction of anesthesia. The treatment group had a significantly lower systemic vascular resistance index (SVRI) at 60 min after medication. No other significant intergroup differences in hemodynamic variables were observed. In addition, significantly more patients in the treatment group required vasopressor therapy. In both groups, the PAP was significantly reduced by general

anesthesia, and almost normalized after valvular heart surgery. The combination of preoperative oral sildenafil and beraprost treatment resulted in a loss of pulmonary selectivity, and did not provide any additional pulmonary vasodilation or benefits perioperatively.

Wang *et al*. (Wang et al., 2009) investigated the postoperative effects of inhaled milrinone in 48 patients with PH undergoing MVR. Patients were randomly assigned to receive inhaled milrinone (nebulized for 4 hours) or intravenous milrinone (control group bolus of 50 microg/kg i.v. milrinone and then received a continuous milrinone infusion, 0.5 microg/kg/min, for 4 hours) After milrinone administration, mPAP and PVR showed a comparable decrease in both groups. However, both mean mPAP and systemic vascular resistance (SVR) in the inhaled group were significantly higher than in the control group. Both mPAP and PVR returned to baseline values 60 minutes after termination of milrinone inhalation. In addition, in the inhaled group, there was a reduction in intrapulmonary shunt fraction (Qs/Qt), with an improvement in arterial oxygen tension/fraction of inspired oxygen ($PaO_2/FiO_2$).

A study by Fattouch *et al*. (Fattouch et al., 2005) evaluated the effects of $iPGI_2$ and iNO and compared them with those of conventional intravenous vasodilators (i.e. NTG and nitroprusside) in 58 patients with PH (PVR >250 dyn·sec·$cm^{-5}$ and mPAP >25 mmHg) suffering from severe mitral valve stenosis. Both drugs were administered by inhalation 5 min before weaning from CPB and continued in the ICU for up to 2 hours. Significant decreases in mPAP and PVR, as well as increases in CO and RVEF, were noted in both inhaled groups, which was not the case in the conventional group. Furthermore, patients in the inhaled groups showed easier separation from CPB, lower requirements for vasoactive drugs and shorter ICU and hospital lengths of stay.

The same investigators also compared the same three strategies in 58 patients with mitral valve stenosis and elevated PVR (>200 dyn·sec·$cm^{-5}$ and/or a transpulmonary gradient (mPAP-PAOP) >10 mmHg) after MVR (Fattouch et al., 2006). Intravenous nitroprusside (5–15 g/min), $iPGI_2$ (10 g/min) or iNO (20 ppm) were started immediately after patient admission to the ICU. Reduction in mPAP, PVR, and transpulmonary gradient were observed in all groups. Only $iPGI_2$ was associated with a significant

increase in stroke volume and CO. Administration of nitroprusside was associated with a reduction in SVR and occurrence of systemic hypotension.

Feneck *et al*. (Feneck et al., 2001) compared milrinone to dobutamine in 120 patients with PAOP >10 mmHg and low output syndrome after CBP (CO <2 L/min/m²). In a subset of patients with PH (PVR >200 dyn·sec·cm$^{-5}$; mPAP >25 mmHg), milrinone and dobutamine had similar effects in reducing PVR and CI. However, milrinone was more effective in reducing PAOP and SVR.

Finally, in 20 patients scheduled for mitral valve surgery with PH (mPAP >25 mmHg), Hachenberg *et al*. (Hachenberg et al., 1997) explored the role of enoximone compared to a combination of NTG and dobutamine, given after induction of anesthesia and then restarted before the end of CPB. Only enoximone was associated with a decrease in mPAP and PVR.

In the presence of PH secondary to LV failure, intra-aortic balloon counterpulsation may facilitate LV recovery.

**Patient-prosthesis mismatch**

In the presence of prosthetic valve dysfunction after CPB, returning under CPB to correct the problem is considered the treatment of choice (Fig. **9**).

## 4.2. Experience at the Montreal Heart Institute

At the Montreal Heart Institute, $iPGI_2$ (Hache et al., 2001; Hache et al., 2003) and inhaled milrinone (Lamarche et al., 2005; Lamarche et al., 2007) are commonly administered to patients when PH and RV dysfunction occur before or after cardiac surgery. Oral sildenafil and iNO are also used in refractory cases in the ICU. Administration by inhalation has the advantage of selectively reaching well-ventilated regions of the lung and thus avoiding undesired decreases of systemic pressures. Future strategies may include the combination of currently available drugs and improvement of methods of administration for current drugs.

# 5. Importance and impact of pulmonary hypertension in cardiac surgery

Preoperative PH is associated with increased morbidity and mortality in cardiac surgery (Tuman et al., 1992; Tremblay et al., 1993; Reich et al., 1999; Bernstein & Parsonnet, 2000; Malouf et al., 2002). Therefore, awareness of PH is very important and its presence in any form should be routinely reported to the surgeon and be evaluated in risk stratification models. Yet, this is not always the case, since only 4/19 risk stratification models in cardiac surgery include preoperative PH as a risk factor (Nilsson et al., 2006). Interestingly, PH is included in the EuroSCORE model which had the greatest discriminatory power over all other models. In a Swedish study including 4351 CABG patients, the receiver operating characteristics (ROC) of the EuroSCORE model was found to be 0.86 and 0.75 for the 30-day and one year mortality rates, respectively.

Analysis performed using the Montreal Heart Institute anesthesia database in 1999 on a total of 1439 patients revealed a mean preoperative sPAP of 31±10 mmHg. PH was defined as sPAP >30 mmHg and was observed in 605 patients (42%). The type of procedures performed in this subpopulation were mainly MVR (*n*=80, 40±14 mmHg), followed by combined CABG and valve procedures (*n*=126, 36±13 mmHg), multiple valve procedures (*n*=60, 36±16 mmHg) and heart transplantations (*n*=6, 36±14 mmHg). Severe PH defined as mAP/mPAP ratio <2 was observed in 16 patients, who all experienced difficult separation from CPB, half of them required postoperative vasoactive support for more than 24 hours while 3 of them died (18.7%).

Thus, PH present before, during or after the operation has an impact on survival mostly through its deleterious effect on right-sided heart function. The most dreaded consequence of PH is the increase in RV afterload and RV dysfunction which will be addressed herein.

## 5.1. Right ventricular dysfunction

Regardless of the underlying cause, uncontrolled PH leads to RV dysfunction. There is growing evidence showing that morbidity and mortality associated with PH depends on RV adaptation to the disease rather than on the absolute values of PAP (D'Alonzo et al.,

1991; Yeo et al., 1998; Ramakrishna et al., 2005; Voelkel et al., 2006; Haddad et al., 2009). Furthermore, studies addressing hemodynamic variables and survival in idiopathic pulmonary arterial hypertension show that high mean Pra and low CO are consistently associated with poorer survival while PAP values are only moderately related to outcome (D'Alonzo et al., 1991; Chin et al., 2005).

Many studies, in a variety of clinical settings, have demonstrated the importance of RV function in cardiac surgery (Table **3**) (Haddad et al., 2009). Typical pathologies and treatments in these studies included high risk coronary or valvular heart disease, congenital heart disease, heart transplantation, patients requiring mechanical assist devices, and unstable patients postoperatively. However, most of the evidence supporting the importance of RV function pertains to retrospective and small prospective studies. Moreover, parameters of RV function have not yet been included in large scale models of risk stratification and thus, their incremental value to the Parsonnet Score and the EuroSCORE has not been well established (Bernstein & Parsonnet, 2000; Nashef et al., 2002; Shroyer et al., 2003; Ambler et al., 2005). A panel in 2006 from the National Institute of Health (NIH) has emphasized the importance of conductingresearch to better understand RV failure (Voelkel et al., 2006).

**Table 3**. Prognostic Value of Right Ventricular Function in Cardiac Surgery (selected studies)

| Study | Population | Study Design | RV dysfunction | Results |
|---|---|---|---|---|
| Reitchert *et al*. | **Unstable post-operative patients** | Prospective<br>*n*=60 | RVFAC <35% | RV dysfunction associated with high mortality rates |
| Pinzani *et al*. | Mitral and combined mitro-aortic surgery | Retrospective<br>*n*=382 | Clinical definition | Postoperative RV failure is the strongest predictor of postoperative mortality |
| Cullen *et al*. | Tetralogy of Fallot | Prospective<br>*n*=35 | Restrictive RV physiology | Restrictive physiology predicts longer intensive care unit stay post repair and lower cardiac output |
| Gatzoulis *et al*. | Tetralogy of Fallot | Prospective<br>*n*=41 | Restrictive RV physiology | Restrictive physiology predicts smaller RV and better exercise tolerance |
| Kromos *et al*. | LVAD and RV failure | Retrospective<br>*n*=31 | Clinical mean RVEF=11.8% | Preoperative clinical factors such as fever, pulmonary edema, and intraoperative blood transfusions were associated with RVAD need |
| Hosenpud *et al*. | Heart Transplantation | Retrospective (International Society for Heart & Lung transplantation)<br>*n*=69,205 | RV failure associated with circulatory failure | RV failure accounts for up to 20% of early deaths |
| Oehiai *et al*. | LVAD | Retrospective<br>*n*=245 | RV failure requiring RVAD | 23 patients (9%) required RVAD. The need for circulatory support, female gender, and non-ischemic etiology were predictors of RVAD need. |
| Maslow *et al*. | CAD undergoing coronary bypass surgery with LVEF <25% | Retrospective<br>*n*=41 | RVFAC <35% | RV dysfunction is associated with decreased long term survival |
| Therrien *et al*. | Tetralogy of Fallot | Prospective<br>*n*=17 | RV remodeling | Severe RV dilatation (RVEDV $\geq$170 ml/m$^2$ or RVESV >85 ml/m$^2$) associated with incomplete RV remodeling |
| Webb *et al*. | Atrial septal defect | Retrospective series | RV remodeling | Older age at repair and abnormal RV myocardial relaxation were associated with incomplete RV remodeling |
| Denault *et al.* | Patients undergoing bypass surgery | Retrospective and prospective<br>*n*=800 | Dynamic obstruction of RVOTO<br>(Gradient >25 mmHg) | Incidence: 4%, dynamic obstruction of RVOTO was associated with a higher incidence of difficult weaning from bypass |
| Haddad *et al*. | High risk valvular surgery | Prospective<br>*n*=50 | RVFAC <32% or RVMPI >0.50 | Preoperative RV dysfunction was associated with a higher incidence of post-operative circulatory failure |

CAD: coronary artery disease; LVEF: left ventricular ejection fraction; LVAD: left ventricular assist device; RV: right ventricular; RVAD: right ventricular assist device; RVESV: right ventricular end-systolic volume; RVEDV: right ventricular end-diastolic volume; RVEF: right ventricular ejection fraction; RVFAC: right ventricular fractional area change; RVMPI: right ventricular myocardial performance index; RVOTO: right ventricular outflow tract obstruction. (Haddad et al., 2009)

### 5.1.1. Before the procedure

In patients with severe aortic stenosis, Boldt *et al.* (Boldt et al., 1992) demonstrated that preoperative RV dysfunction was associated with increased requirements for postoperative inotropic support.

In a retrospective study involving patients undergoing mitral and mitral-aortic valvular surgery, Pinzani *et al*. (Pinzani et al., 1993) showed that preoperative RV failure was associated with increased perioperative mortality. Furthermore, in that study, postoperative RV failure was the most important independent predictor of late survival.

In a small prospective study of 14 patients with severe non-ischemic mitral regurgitation presenting high risk descriptors (LV ejection fraction (LVEF) ≤ 45% or RVEF ≤ 20%), Wencker *et al*. (Wencker et al., 2000) found that preoperative RVEF ≤ 20% predicted late postoperative death.

In a retrospective study of 41 patients undergoing non-emergent coronary artery bypass surgery, Maslow *et al.* (Maslow et al., 2002) have shown than RV dysfunction (right ventricular fractional area change (RVFAC) <35%) in presence of severe LV dysfunction (LVEF ≤25%) was associated with an increased risk of postoperative morbidity and mortality. Furthermore, patients with RV dysfunction presented a higher prevalence of diabetes mellitus and renal disease, a higher incidence of postoperative support (inotropic or mechanical), longer ICU and hospital stays, as well as a decrease in short and long term survival.

**Experience at the Montreal Heart Institute**

Haddad *et al.* (Haddad et al., 2007) further assessed the value of RV function relative to other validated risk factors in open valvular heart surgery on 50 patients undergoing valvular surgery. Patients with RV myocardial performance index (RVMPI) <50% (*n*=20) presented a significantly higher occurrence of circulatory failure (16/20 (80%) *vs* 6/30 (20%), p <0.0001) as well as a higher postoperative heart failure mortality (14/20 (74%) *vs* 3/30 (10%), p <0.0001). In addition, multivariate analysis revealed RVMPI as the only independent predictor of heart failure and mortality among all other demographic, hemodynamic and echocardiographic variables (p <0.0001).

### 5.1.2. After the procedure

Right ventricular failure after CPB is associated with a mortality rate ranging from 44% to 86% (Davila-Roman et al., 1995). The incidence of acute refractory RV failure ranges from 0.04 to 0.1% after cardiac surgery. Acute refractory RV failure has also been reported in 2-3% patients after heart transplantation and in 20-30% patients receiving support from a LV assist device with a reported initial salvage rate as low as 25-30% (Kaul & Fields, 2000).

## 5.2. Treatment of right ventricular failure

A proposed algorithm for the treatment of RV failure used at the Montreal Heart Institute is summarised in Fig. **10**. Assessment of RV function is performed visually when the chest is opened, by analysing RV pressure waveforms and using transesophageal echocardiography. Once RVOTO is ruled out, the etiology of RV systolic dysfunction is divided in two categories, either ischemic or not and with or without LV failure. If ischemia is suspected of causing either RV failure or bi-ventricular failure, treatments (medical and surgical) will be oriented towards the promotion of RV perfusion by means of thrombolysis, percutaneous transluminal coronary angioplasty, or CABG. Finally, pulmonary artery balloon pump, RV assist device or cavopulmonary diversion have also been described as potential treatments for severe RV dysfunction (Kaul & Fields, 2000). Otherwise, if a non-ischemic etiology is more likely or if no LV failure is present, treatments will rather be oriented towards an increase in contractility (inotropes) and a reduction in RV afterload (iNO, $iPGI_2$, inhaled milrinone, oral sildenafil).

Optimizing oxygenation and ventilation and ruling out other reversible causes of reduction in venous return such as reduction in mAP, increase in Pra and increase in resistance to venous return will also be important in managing these patients.

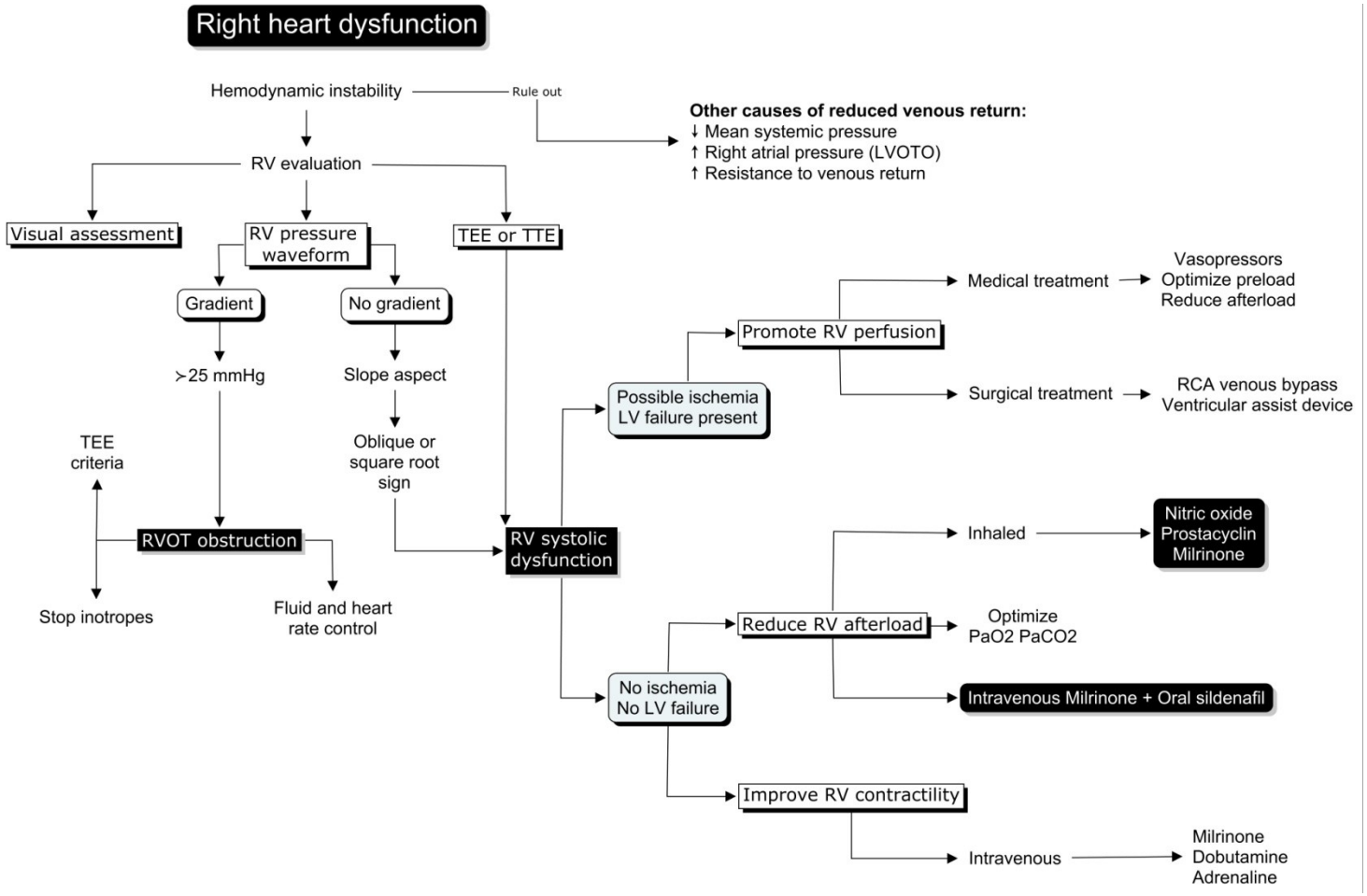


**Figure 10**. Proposed approach in the treatment of right ventricular (RV) dysfunction. (LVOTO: left ventricular outflow tract obstruction; RCA: right coronary artery; RVOTO: right ventricular outflow tract obstruction; TEE: transesophageal echocardiography; TTE: transthoracic echocardiography). Presented at the 2011 Canadian Anesthesiologist Society Annual Meeting in Toronto, ON, Canada.

# 6. Prevention of pulmonary hypertension in cardiac surgery

## 6.1. Pharmacological

Prevention of PH represents a promising strategy to prevent RV failure, its most important consequence after cardiac surgery. To date, very few studies have addressed this issue and one of the potential avenues constitutes the prevention of the pulmonary reperfusion syndrome. In this regard, both $iPGI_2$ (Fortier et al., 2004) and inhaled milrinone (Lamarche et al., 2005) have been demonstrated to prevent CPB-induced endothelial dysfunction, in an animal model. A pilot randomized control trial (RCT) conducted by Hache *et al.* (Hache et al., 2003) in patients with preoperative PH concluded that $iPGI_2$ was superior to placebo in reducing PH and was also associated with lower requirements for vasoactive support.

A pilot RCT was conducted on the administration of inhaled milrinone before CPB in 21 patients, all undergoing valvular surgeries (Denault et al., 2010b). Procedures consisted of 14 complex surgeries and 5 reoperations. The study included a total of 8 males and 13 females with a mean age of 70±6.3 years old and a mean Parsonnet Score of 32±9. Inhaled milrinone ($n$=10) significantly reduced mean sPAP, which decreased from 66±20 mmHg (pre-CPB) to 46±20 mmHg (after CPB) ($p < 0.001$). In contrast, sPAP remained unchanged in the control group ($n$=11) and no significant differences between groups were observed in decreased systemic arterial pressures.

A retrospective study reporting the preliminary experience on the use of inhaled milrinone at the Montreal Heart Institute was conducted in 70 high risk patients with a mean Parsonnet Score of 27±14 (Bernstein & Parsonnet, 2000; Lamarche et al., 2007). Results were compared to those of a control group with similar baseline characteristics. In conclusion, the administration of inhaled milrinone prior to CPB ($n$=30) was associated with a lower chance of CPB re-initiation (9 *vs* 1; p=0.021) and lower postoperative PAP. Further studies (#NCT00819377) are underway to determine the efficacy of this approach.

### 6.2. Non-pharmacological

In addition to therapeutic approaches to the prevention of PH, the choice of type and size of aortic prosthetic valve may be a very important factor. As previously discussed, it has been shown that, if the EOA of the aortic valve is too small relative to body size, the so-called PPM, the intraoperative and long-term mortality will increase (Milano et al., 2001; Rao et al., 2000; Pibarot & Dumesnil, 2000; Blais et al., 2003; Ruel et al., 2004; Pibarot & Dumesnil, 2006; Tasca et al., 2006; Kulik et al., 2006). Hence, prevention of PPM may contribute to reducing PH after cardiac surgery and facilitate separation from CPB. This includes strategies such as the implantation of a prosthesis with better performance (stentless bioprosthesis, new generation bileaflet mechanical valve, new generation supra-annular stented bioprosthetic valve) or enlargement of the aortic root (Fig. **9**) in order to accommodate a larger prosthesis. On the other hand, some strategies used to prevent PPM are complex and may even increase the risk of difficult weaning from CPB extending the duration of the surgical procedure and consequently, CPB duration. Unfortunately, in some cases, the drawbacks of using alternative procedures may supercede the benefits of avoiding PPM. Therefore, the establishment of accurate criteria for a better assessment of the benefit-risk ratio with respect to the prevention of PPM is essential. In the case of mitral valve PPM, the best option would be to favor mitral valve repair rather than replacement. However, mitral valve repair may not be possible in a significant number of patients, which limits the options when compared to aortic valve replacement (Magne et al., 2007).

## 7. Conclusion

Pulmonary hypertension and its most dreaded consequence, RV dysfunction, are important mortality risk factors in cardiac surgery. Accordingly, all cardiac patients may benefit from early diagnosis and/or treatment prior to the surgical procedure. In patients with PH, further evaluation of potential alterations in the RV function would be relevant. Thus, future trials should prioritize in-depth exploration of preventive approaches in order to address the role of preemptive reduction of PH severity before cardiac surgery and to determine its impact on postoperative outcomes and survival improvement.

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# 8. References


Ambler, G., Omar, R. Z., Royston, P., Kinsman, R., Keogh, B. E., & Taylor, K. M. (2005). Generic, simple risk stratification model for heart valve surgery. *Circulation*. 112, 2, 224-231, ISSN 1524-4539

Asimakopoulos, G., Smith, P. L., Ratnatunga, C. P., & Taylor, K. M. (1999). Lung injury and acute respiratory distress syndrome after cardiopulmonary bypass. *Ann Thorac Surg*. 68, 3, 1107-1115, ISSN 0003-4975

Bakhtiary, F., Schiemann, M., Dzemali, O., Dogan, S., Schachinger, V., Ackermann, H., Moritz, A., & Kleine, P. (2007). Impact of patient-prosthesis mismatch and aortic valve design on coronary flow reserve after aortic valve replacement. *J Am Coll Cardiol*. 49, 7, 790-796, ISSN 1558-3597

Bernstein, A. D. & Parsonnet, V. (2000). Bedside estimation of risk as an aid for decision-making in cardiac surgery. *Ann Thorac Surg*. 69, 3, 823-828, ISSN 0003-4975

Bjornsson, J. & Edwards, W. D. (1985). Primary pulmonary hypertension: a histopathologic study of 80 cases. *Mayo Clin Proc*. 60, 1, 16-25, ISSN 0025-6196

Blais, C., Dumesnil, J. G., Baillot, R., Simard, S., Doyle, D., & Pibarot, P. (2003). Impact of valve prosthesis-patient mismatch on short-term mortality after aortic valve replacement. *Circulation*. 108, 8, 983-988, ISSN 1524-4539

Boldt, J., Zickmann, B., Ballesteros, M., Dapper, F., & Hempelmann, G. (1992). Right ventricular function in patients with aortic stenosis undergoing aortic valve replacement. *J Cardiothorac Vasc Anesth*. 6, 3, 287-291, ISSN 1053-0770

Chin, K. M., Kim, N. H., & Rubin, L. J. (2005). The right ventricle in pulmonary hypertension. *Coron Artery Dis*. 16, 1, 13-18, ISSN 0954-6928

D'Alonzo, G. E., Barst, R. J., Ayres, S. M., Bergofsky, E. H., Brundage, B. H., Detre, K. M., Fishman, A. P., Goldring, R. M., Groves, B. M., & Kernis, J. T. (1991). Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med*. 115, 5, 343-349, ISSN 0003-4819

Dartevelle, P., Fadel, E., Mussot, S., Chapelier, A., Herve, P., de Perrot, M., Cerrina, J., Ladurie, F. L., Lehouerou, D., Humbert, M., Sitbon, O., & Simonneau, G. (2004). Chronic thromboembolic pulmonary hypertension. *Eur Respir J*. 23, 4, 637-648, ISSN 0903-1936

Davila-Roman, V. G., Waggoner, A. D., Hopkins, W. E., & Barzilai, B. (1995). Right ventricular dysfunction in low output syndrome after cardiac operations: assessment by transesophageal echocardiography. *Ann Thorac Surg*. 60, 4, 1081-1086, ISSN 0003-4975

Delgado, J. F., Conde, E., Sanchez, V., Lopez-Rios, F., Gomez-Sanchez, M. A., Escribano, P., Sotelo, T., Gomez, d. l. C., Cortina, J., & de la Calzada, C. S.

(2005). Pulmonary vascular remodeling in pulmonary hypertension due to chronic heart failure. *Eur J Heart Fail*. 7, 6, 1011-1016, ISSN 1388-9842

Denault, A. Y., Couture, P., Vegas, A., Buithieu, J., & Tardif, J. C. (2010a). *Transesophageal Echocardiography Multimedia Manual: A Perioperative Transdisciplinary Approach,* (Second Edition), Informa Healthcare, ISBN 9781-420-08070-4, New York

Denault, A. Y., Haddad, F., Lamarche, Y., Nguyen, A. Q. N., Varin, F., Levesque, S., Shi, Y., Perrault, L. P., Tardif, J. C., and Lambert, J. (2010b). Inhaled Milrinone in Cardiac Surgery, *Procedings of Canadian Anesthesiologists' Society*, Montreal, QC, Canada, June 2010b

Downing, S. W. & Edmunds, L. H., Jr. (1992). Release of vasoactive substances during cardiopulmonary bypass. *Ann Thorac Surg*. 54, 6, 1236-1243, ISSN 0003-4975

Fattouch, K., Sbraga, F., Bianco, G., Speziale, G., Gucciardo, M., Sampognaro, R., & Ruvolo, G. (2005). Inhaled prostacyclin, nitric oxide, and nitroprusside in pulmonary hypertension after mitral valve replacement. *J Card Surg*. 20, 2, 171-176, ISSN 0886-0440

Fattouch, K., Sbraga, F., Sampognaro, R., Bianco, G., Gucciardo, M., Lavalle, C., Vizza, C. D., Fedele, F., & Ruvolo, G. (2006). Treatment of pulmonary hypertension in patients undergoing cardiac surgery with cardiopulmonary bypass: a randomized, prospective, double-blind study. *J Cardiovasc Med (Hagerstown )*. 7, 2, 119-123, ISSN 1558-2027

Feneck, R. O., Sherry, K. M., Withington, P. S., & Oduro-Dominah, A. (2001). Comparison of the hemodynamic effects of milrinone with dobutamine in patients after cardiac surgery. *J Cardiothorac Vasc Anesth*. 15, 3, 306-315, ISSN 1053-0770

Fernandes, J. L., Sampaio, R. O., Brandao, C. M., Accorsi, T. A., Cardoso, L. F., Spina, G. S., Tarasoutchi, F., Pomerantzeff, P., Auler, J. O., Jr., & Grinberg, M. (2011). Comparison of inhaled nitric oxide versus oxygen on hemodynamics in patients with mitral stenosis and severe pulmonary hypertension after mitral valve surgery. *Am J Cardiol*. 107, 7, 1040-1045

Fischer, L. G., Van, A. H., & Burkle, H. (2003). Management of pulmonary hypertension: physiological and pharmacological considerations for anesthesiologists. *Anesth Analg*. 96, 6, 1603-1616, ISSN 0003-2999

Fortier, S., DeMaria, R. G., Lamarche, Y., Malo, O., Denault, A. Y., Desjardins, F., Carrier, M., & Perrault, L. P. (2004). Inhaled prostacyclin reduces cardiopulmonary bypass-induced pulmonary endothelial dysfunction via increased cyclic adenosine monophosphate levels. *J Thorac Cardiovasc Surg*. 128, 1, 109-116, ISSN 0022-5223

Fuster, V., Steele, P. M., Edwards, W. D., Gersh, B. J., McGoon, M. D., & Frye, R. L. (1984). Primary pulmonary hypertension: natural history and the importance of thrombosis. *Circulation*. 70, 4, 580-587, ISSN 0009-7322

Gomez, C. M. & Palazzo, M. G. (1998). Pulmonary artery catheterization in anaesthesia and intensive care. *Br J Anaesth*. 81, 6, 945-956, ISSN 0007-0912

Hache, M., Denault, A. Y., Belisle, S., Couture, P., Babin, D., Tetrault, F., & Guimond, J. G. (2001). Inhaled prostacyclin (PGI2) is an effective addition to the treatment of pulmonary hypertension and hypoxia in the operating room and intensive care unit. *Can J Anaesth*. 48, 9, 924-929, ISSN 0832-610X

Hache, M., Denault, A. Y., Belisle, S., Robitaille, D., Couture, P., Sheridan, P., Pellerin, M., Babin, D., Noel, N., Guertin, M. C., Martineau, R., & Dupuis, J. (2003). Inhaled epoprostenol (prostacyclin) and pulmonary hypertension before cardiac surgery. *J Thorac Cardiovasc Surg*. 125, 3, 642-649, ISSN 0022-5223

Hachenberg, T., Mollhoff, T., Holst, D., Hammel, D., & Brussel, T. (1997). Cardiopulmonary effects of enoximone or dobutamine and nitroglycerin on mitral valve regurgitation and pulmonary venous hypertension. *J Cardiothorac Vasc Anesth*. 11, 4, 453-457, ISSN 1053-0770

Haddad, F., Couture, P., Tousignant, C., & Denault, A. Y. (2009). The right ventricle in cardiac surgery, a perioperative perspective: II. Pathophysiology, clinical importance, and management. *Anesth Analg*. 108, 2, 422-433

Haddad, F., Denault, A. Y., Couture, P., Cartier, R., Pellerin, M., Levesque, S., Lambert, J., & Tardif, J. C. (2007). Right ventricular myocardial performance index predicts perioperative mortality or circulatory failure in high-risk valvular surgery. *J Am Soc Echocardiogr*. 20, 9, 1065-1072

Humbert, M., Sitbon, O., & Simonneau, G. (2004). Treatment of pulmonary arterial hypertension. *N Engl J Med*. 351, 14, 1425-1436

Jais, X., D'Armini, A. M., Jansa, P., Torbicki, A., Delcroix, M., Ghofrani, H. A., Hoeper, M. M., Lang, I. M., Mayer, E., Pepke-Zaba, J., Perchenet, L., Morganti, A., Simonneau, G., & Rubin, L. J. (2008). Bosentan for treatment of inoperable chronic thromboembolic pulmonary hypertension: BENEFiT (Bosentan Effects in iNopErable Forms of chronIc Thromboembolic pulmonary hypertension), a randomized, placebo-controlled trial. *J Am Coll Cardiol*. 52, 25, 2127-2134, ISSN 1558-3597

Jamieson, S. W., Kapelanski, D. P., Sakakibara, N., Manecke, G. R., Thistlethwaite, P. A., Kerr, K. M., Channick, R. N., Fedullo, P. F., & Auger, W. R. (2003). Pulmonary endarterectomy: experience and lessons learned in 1,500 cases. *Ann Thorac Surg*. 76, 5, 1457-1462

Jamieson, S. W. & Nomura, K. (2000). Indications for and the results of pulmonary thromboendarterectomy for thromboembolic pulmonary hypertension. *Semin Vasc Surg*. 13, 3, 236-244

Kaul, T. K. & Fields, B. L. (2000). Postoperative acute refractory right ventricular failure: incidence, pathogenesis, management and prognosis. *Cardiovasc Surg*. 8, 1, 1-9

Khan, T. A., Schnickel, G., Ross, D., Bastani, S., Laks, H., Esmailian, F., Marelli, D., Beygui, R., Shemin, R., Watson, L., Vartapetian, I., & Ardehali, A. (2009). A prospective, randomized, crossover pilot study of inhaled nitric oxide versus inhaled prostacyclin in heart transplant and lung transplant recipients. *J Thorac Cardiovasc Surg*. 138, 6, 1417-1424

Kim, S. Y., Shim, J. K., Shim, Y. H., Hong, S. W., Choi, K. H., & Kwak, Y. L. (2010). Sildenafil and beraprost combination therapy in patients with pulmonary hypertension undergoing valvular heart surgery. *J Heart Valve Dis*. 19, 3, 333-340

Kulik, A., Burwash, I. G., Kapila, V., Mesana, T. G., & Ruel, M. (2006). Long-term outcomes after valve replacement for low-gradient aortic stenosis: impact of prosthesis-patient mismatch. *Circulation*. 114, 1 Suppl, I553-I558

Lamarche, Y., Malo, O., Thorin, E., Denault, A. Y., Carrier, M., Roy, J., & Perrault, L. P. (2005). Inhaled but not intravenous milrinone prevents pulmonary endothelial dysfunction after cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. 130, 1, 83-92

Lamarche, Y., Perrault, L. P., Maltais, S., Tetreault, K., Lambert, J., & Denault, A. Y. (2007). Preliminary experience with inhaled milrinone in cardiac surgery. *Eur J Cardiothorac Surg*. 31, 6, 1081-1087

Lesage, A. M., Tsuchioka, H., Young, W. G., Jr., & Sealy, W. C. (1966). Pathogenesis of pulmonary damage during extracorporeal perfusion. *Arch Surg*. 93, 6, 1002-1008

Magne, J., Mathieu, P., Dumesnil, J. G., Tanne, D., Dagenais, F., Doyle, D., & Pibarot, P. (2007). Impact of Prosthesis-Patient Mismatch on Survival After Mitral Valve Replacement. *Circulation*. 115, 11, 1417-1425

Malouf, J. F., Enriquez-Sarano, M., Pellikka, P. A., Oh, J. K., Bailey, K. R., Chandrasekaran, K., Mullany, C. J., & Tajik, A. J. (2002). Severe pulmonary hypertension in patients with severe aortic valve stenosis: clinical profile and prognostic implications. *J Am Coll Cardiol*. 40, 4, 789-795

Martineau, A., Arcand, G., Couture, P., Babin, D., Perreault, L. P., & Denault, A. Y. (2003). Transesophageal echocardiographic diagnosis of carbon dioxide embolism during minimally invasive saphenous vein harvesting and treatment with inhaled epoprostenol. *Anesth Analg*. 96, 4, 962-964

Maslow, A. D., Regan, M. M., Panzica, P., Heindel, S., Mashikian, J., & Comunale, M. E. (2002). Precardiopulmonary bypass right ventricular function is associated with poor outcome after coronary artery bypass grafting in patients with severe left ventricular systolic dysfunction. *Anesth Analg*. 95, 6, 1507-1518

McLaughlin, V. V. & McGoon, M. D. (2006). Pulmonary arterial hypertension. *Circulation*. 114, 13, 1417-1431

Milano, A. D., Blanzola, C., Mecozzi, G., D'Alfonso, A., De Carlo, M., Nardi, C., & Bortolotti, U. (2001). Hemodynamic performance of stented and stentless aortic bioprostheses. *Ann Thorac Surg*. 72, 1, 33-38

Moher, D., Schulz, K. F., & Altman, D. G. (2001). The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Lancet*. 357, 9263, 1191-1194

Moraes, D. L., Colucci, W. S., & Givertz, M. M. (2000). Secondary pulmonary hypertension in chronic heart failure: the role of the endothelium in pathophysiology and management. *Circulation*. 102, 14, 1718-1723, ISSN 1524-4539

Nashef, S. A., Roques, F., Hammill, B. G., Peterson, E. D., Michel, P., Grover, F. L., Wyse, R. K., & Ferguson, T. B. (2002). Validation of European System for Cardiac Operative Risk Evaluation (EuroSCORE) in North American cardiac surgery. *Eur J Cardiothorac Surg*. 22, 1, 101-105

Nilsson, J., Algotsson, L., Hoglund, P., Luhrs, C., & Brandt, J. (2006). Comparison of 19 pre-operative risk stratification models in open-heart surgery. *Eur Heart J*. 27, 7, 867-874

Ocal, A., Kiris, I., Erdinc, M., Peker, O., Yavuz, T., & Ibrisim, E. (2005). Efficiency of prostacyclin in the treatment of protamine-mediated right ventricular failure and acute pulmonary hypertension. *Tohoku J Exp Med*. 207, 1, 51-58

Oudiz, R. J. (2007). Pulmonary hypertension associated with left-sided heart disease. *Clin Chest Med*. 28, 1, 233-41, x, ISSN 0272-5231

Pengo, V., Lensing, A. W., Prins, M. H., Marchiori, A., Davidson, B. L., Tiozzo, F., Albanese, P., Biasiolo, A., Pegoraro, C., Iliceto, S., & Prandoni, P. (2004). Incidence of chronic thromboembolic pulmonary hypertension after pulmonary embolism. *N Engl J Med*. 350, 22, 2257-2264, ISSN 1533-4406

Pibarot, P. & Dumesnil, J. G. (2000). Hemodynamic and clinical impact of prosthesis-patient mismatch in the aortic valve position and its prevention. *J Am Coll Cardiol*. 36, 4, 1131-1141

Pibarot, P. & Dumesnil, J. G. (2006). Prosthesis-patient mismatch: definition, clinical impact, and prevention. *Heart*. 92, 8, 1022-1029

Pinzani, A., de Gevigney, G., Pinzani, V., Ninet, J., Milon, H., & Delahaye, J. P. (1993). [Pre- and postoperative right cardiac insufficiency in patients with mitral or mitral-aortic valve diseases]. *Arch Mal Coeur Vaiss*. 86, 1, 27-34

Ramakrishna, G., Sprung, J., Ravi, B. S., Chandrasekaran, K., & McGoon, M. D. (2005). Impact of pulmonary hypertension on the outcomes of noncardiac surgery: predictors of perioperative morbidity and mortality. *J Am Coll Cardiol*. 45, 10, 1691-1699

Rao, V., Jamieson, W. R., Ivanov, J., Armstrong, S., & David, T. E. (2000). Prosthesis-patient mismatch affects survival after aortic valve replacement. *Circulation*. 102, 19 Suppl 3, III5-III9

Reich, D. L., Bodian, C. A., Krol, M., Kuroda, M., Osinski, T., & Thys, D. M. (1999). Intraoperative hemodynamic predictors of mortality, stroke, and myocardial infarction after coronary artery bypass surgery. *Anesth Analg*. 89, 4, 814-822

Rich, S., Kaufmann, E., & Levy, P. S. (1992). The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. *N Engl J Med*. 327, 2, 76-81, ISSN 0028-4793

Robitaille, A., Denault, A. Y., Couture, P., Belisle, S., Fortier, A., Guertin, M. C., Carrier, M., & Martineau, R. (2006). Importance of relative pulmonary hypertension in cardiac surgery: the mean systemic-to-pulmonary artery pressure ratio. *J Cardiothorac Vasc Anesth*. 20, 3, 331-339

Rubin, L. J., Hoeper, M. M., Klepetko, W., Galie, N., Lang, I. M., & Simonneau, G. (2006). Current and future management of chronic thromboembolic pulmonary hypertension: from diagnosis to treatment responses. *Proc Am Thorac Soc*. 3, 7, 601-607, ISSN 1546-3222

Rubin, L. J. & Rich, S. (1997). *Primary pulmonary hypertension,* M. Dekker, ISBN 0824795059, New York

Ruel, M., Rubens, F. D., Masters, R. G., Pipe, A. L., Bedard, P., Hendry, P. J., Lam, B. K., Burwash, I. G., Goldstein, W. G., Brais, M. P., Keon, W. J., & Mesana, T. G. (2004). Late incidence and predictors of persistent or recurrent heart failure in patients with aortic prosthetic valves. *J Thorac Cardiovasc Surg*. 127, 1, 149-159

Sackett, D. L. (1989). Rules of evidence and clinical recommendations on the use of antithrombotic agents. *Chest*. 95, 2 Suppl, 2S-4S

Schmid, E. R., Burki, C., Engel, M. H., Schmidlin, D., Tornic, M., & Seifert, B. (1999). Inhaled nitric oxide versus intravenous vasodilators in severe pulmonary hypertension after cardiac surgery. *Anesth Analg*. 89, 5, 1108-1115

Shroyer, A. L., Coombs, L. P., Peterson, E. D., Eiken, M. C., DeLong, E. R., Chen, A., Ferguson, T. B., Jr., Grover, F. L., & Edwards, F. H. (2003). The Society of Thoracic Surgeons: 30-day operative mortality and morbidity risk models. *Ann Thorac Surg*. 75, 6, 1856-1864

Simonneau, G., Robbins, I. M., Beghetti, M., Channick, R. N., Delcroix, M., Denton, C. P., Elliott, C. G., Gaine, S. P., Gladwin, M. T., Jing, Z. C., Krowka, M. J., Langleben, D., Nakanishi, N., & Souza, R. (2009). Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol*. 54, 1 Suppl, S43-S54, ISSN 1558-3597

Solina, A., Papp, D., Ginsberg, S., Krause, T., Grubb, W., Scholz, P., Pena, L. L., & Cody, R. (2000). A comparison of inhaled nitric oxide and milrinone for the treatment of pulmonary hypertension in adult cardiac surgery patients. *J Cardiothorac Vasc Anesth*. 14, 1, 12-17

Solina, A. R., Ginsberg, S. H., Papp, D., Grubb, W. R., Scholz, P. M., Pantin, E. J., Cody, R. P., & Krause, T. J. (2001). Dose response to nitric oxide in adult cardiac surgery patients. *J Clin Anesth*. 13, 4, 281-286

Stafford-Smith, M., Lefrak, E. A., Qazi, A. G., Welsby, I. J., Barber, L., Hoeft, A., Dorenbaum, A., Mathias, J., Rochon, J. J., & Newman, M. F. (2005). Efficacy and safety of heparinase I versus protamine in patients undergoing coronary artery bypass grafting with and without cardiopulmonary bypass. *Anesthesiology*. 103, 2, 229-240

Sukernik, M. R., Mets, B., & Bennett-Guerrero, E. (2001). Patent foramen ovale and its significance in the perioperative period. *Anesth Analg*. 93, 5, 1137-1146

Suntharalingam, J., Treacy, C. M., Doughty, N. J., Goldsmith, K., Soon, E., Toshner, M. R., Sheares, K. K., Hughes, R., Morrell, N. W., & Pepke-Zaba, J. (2008). Long-term use of sildenafil in inoperable chronic thromboembolic pulmonary hypertension. *Chest*. 134, 2, 229-236, ISSN 0012-3692

Tapson, V. F. & Humbert, M. (2006). Incidence and prevalence of chronic thromboembolic pulmonary hypertension: from acute to chronic pulmonary embolism. *Proc Am Thorac Soc*. 3, 7, 564-567, ISSN 1546-3222

Tasca, G., Mhagna, Z., Perotti, S., Centurini, P. B., Sabatini, T., Amaducci, A., Brunelli, F., Cirillo, M., DallaTomba, M., Quaini, E., Troise, G., & Pibarot, P. (2006). Impact of prosthesis-patient mismatch on cardiac events and midterm mortality after aortic valve replacement in patients with pure aortic stenosis. *Circulation*. 113, 4, 570-576

Therrien, J., Dore, A., Gersony, W., Iserin, L., Liberthson, R., Meijboom, F., Colman, J. M., Oechslin, E., Taylor, D., Perloff, J., Somerville, J., & Webb, G. D. (2001a). CCS Consensus Conference 2001 update: recommendations for the management of adults with congenital heart disease. Part I. *Can J Cardiol*. 17, 9, 940-959

Therrien, J., Gatzoulis, M., Graham, T., Bink-Boelkens, M., Connelly, M., Niwa, K., Mulder, B., Pyeritz, R., Perloff, J., Somerville, J., & Webb, G. D. (2001b). Canadian Cardiovascular Society Consensus Conference 2001 update: Recommendations for the Management of Adults with Congenital Heart Disease--Part II. *Can J Cardiol*. 17, 10, 1029-1050

Tremblay, N. A., Hardy, J. F., Perrault, J., & Carrier, M. (1993). A simple classification of the risk in cardiac surgery: the first decade. *Can J Anaesth*. 40, 2, 103-111

Tuman, K. J., McCarthy, R. J., March, R. J., Najafi, H., & Ivankovich, A. D. (1992). Morbidity and duration of ICU stay after cardiac surgery. A model for preoperative risk assessment. *Chest*. 102, 1, 36-44

Viaro, F., Dalio, M. B., & Evora, P. R. (2002). Catastrophic cardiovascular adverse reactions to protamine are nitric oxide/cyclic guanosine monophosphate dependent and endothelium mediated: should methylene blue be the treatment of choice? *Chest*. 122, 3, 1061-1066

Voelkel, N. F., Quaife, R. A., Leinwand, L. A., Barst, R. J., McGoon, M. D., Meldrum, D. R., Dupuis, J., Long, C. S., Rubin, L. J., Smart, F. W., Suzuki, Y. J., Gladwin, M., Denholm, E. M., & Gail, D. B. (2006). Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. *Circulation*. 114, 17, 1883-1891

Wan, S., LeClerc, J. L., & Vincent, J. L. (1997). Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. *Chest*. 112, 3, 676-692

Wang, H., Gong, M., Zhou, B., & Dai, A. (2009). Comparison of inhaled and intravenous milrinone in patients with pulmonary hypertension undergoing mitral valve surgery. *Adv Ther*. 26, 4, 462-468

Wencker, D., Borer, J. S., Hochreiter, C., Devereux, R. B., Roman, M. J., Kligfield, P., Supino, P., Krieger, K., & Isom, O. W. (2000). Preoperative predictors of late postoperative outcome among patients with nonischemic mitral regurgitation with 'high risk' descriptors and comparison with unoperated patients. *Cardiology*. 93, 1-2, 37-42

Yeo, T. C., Dujardin, K. S., Tei, C., Mahoney, D. W., McGoon, M. D., & Seward, J. B. (1998). Value of a Doppler-derived index combining systolic and diastolic time intervals in predicting outcome in primary pulmonary hypertension. *Am J Cardiol*. 81, 9, 1157-1161

# 9. Abbreviations

| | |
|---|---|
| Ao | aorta |
| AVR | aortic valve replacement |
| CABG | coronary artery bypass graft |
| CAD | coronary artery disease |
| CI | cardiac index |
| CO | cardiac output |
| COPD | chronic obstructive pulmonary disease |
| CPB | cardiopulmonary bypass |
| CTEPH | chronic thromboembolic pulmonary hypertension |
| CVP | central venous pressure |
| D | diastolic |
| dPAP | diastolic pulmonary arterial pressure |
| EKG | electrocardiogram |
| EOA | effective orifice area |
| ET-1 | Endothelin-1 |
| FRC | functional residual capacity |
| ICU | intensive care unit |
| iNO | inhaled nitric oxide |
| iPGI2 | inhaled prostacyclin |
| LA | left atrium |
| LV | left ventricle or left ventricular |
| LVAD | left ventricular assist device |
| LVEF | left ventricular ejection fraction |
| LVOTO | left ventricular outflow tract obstruction |
| mAP | mean arterial pressure |
| mPAP | mean pulmonary artery pressure |
| MVR | mitral valve replacement |
| NIH | National Institutes of Health |
| NO | nitric oxide |
| NTG | nitroglycerin |

| | |
|---|---|
| $PaO_2/FiO_2$ | arterial oxygen tension/fraction inspired oxygen |
| PAOP | pulmonary artery occlusion pressure |
| PAP | pulmonary artery pressure |
| PCO2 | partial pressure of carbon dioxide |
| PCWP | pulmonary capillary wedge pressure |
| PEEP | positive end-expiratory pressure |
| PFO | patent foramen ovale |
| PG | pressure gradient |
| $PGE_1$ | prostaglandin $E_1$ |
| $PGI_2$ | prostacyclin |
| PH | pulmonary hypertension |
| Pa | arterial pressure |
| Ppa | pulmonary artery pressure |
| PPM | patient-prosthesis mismatch |
| Pra | right atrial pressure |
| Prv | right ventricular pressure |
| PVR | pulmonary vascular resistance |
| PVRI | indexed pulmonary vascular resistance |
| Qs/Qt | intrapulmonary shunt fraction |
| RA | right atrium |
| RCA | right coronary artery |
| RCT | randomized controlled trial |
| ROC | receiver operating characteristics |
| RPA | right pulmonary artery |
| RV | right ventricle or right ventricular |
| RVAD | right ventricular assist device |
| RVEDV | right ventricular end-diastolic volume |
| RVEF | right ventricular ejection fraction |
| RVESV | right ventricular end-systolic volume |
| RVFAC | right ventricular fractional area change |
| RVMPI | right ventricular myocardial performance index |
| RVOTO | right ventricular outflow tract obstruction |

| | |
|---|---|
| RVSP | right ventricular systolic pressure |
| S | systolic |
| sAP | systemic arterial pressure |
| sPAP | systolic pulmonary artery pressure |
| SVR | systemic vascular resistance |
| SVRI | indexed systemic vascular resistance |
| TEE | transesophageal echocardiography |
| TLC | total lung capacity |
| TTE | transthoracic echocardiography |
| Tr | tricuspid regurgitation |
| UK | United Kingdom |
| USA | United States of America |
| V | velocity |
| WHO | World Health Organization |

# ANNEXE II. Manuscrit n°5 (coauteur) A specific and sensitive HPLC-MS/MS micromethod for milrinone plasma levels determination after inhalation in cardiac patients

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**Short title:** HPLC-MS/MS micromethod for plasma milrinone in cardiac patients

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# Abstract

**Background:** Milrinone administered through inhalation is an emerging method aimed at specifically reducing pulmonary hypertension without affecting systemic pressures. Its administration has been shown useful both in patients undergoing cardiac surgery and for persistent pulmonary hypertension of the newborn. These populations are prone to receive many concomitant medications and/or blood sampling may require a low volume quantification method. In order to address these issues in view of pharmacokinetic studies, this paper aims to develop and validate a specific and sensitive analytical assay using HPLC and MS/MS detection for the quantification of milrinone plasma concentrations after inhalation in patients undergoing cardiac surgery.

**Methods and Materials:** Plasma samples (50 µL) were extracted using ethyl acetate. Milrinone was separated on a C18 analytical column at 50°C. The mobile phase consisted of methanol and 10 mM ammonium acetate (45:55 v/v). The electrospray was operated in the negative ionization mode and monitored the following mass transitions: m/z 212.1 → 140.0 at 36 eV for milrinone and m/z 252.1 → 156.1 at 32 eV for olprinone.

**Results:** Calibration curves followed a quadratic regression in the concentration range of 0.3125–640 ng/mL. The lower limit of quantification is 0.3125 ng/mL and is based on a low plasma volume of 50 µL. Mean drug recovery and accuracy were ≥ 72.3% and 96.0%, respectively. Intra- and inter-day precision (CV%) was ≤ 7.4 % and ≤ 11.5%, respectively. The specificity allowed milrinone quantification in the multidrug administration conditions of cardiopulmonary bypass.

**Conclusions:** This validated micromethod proved to be highly sensitive and specific while using a low volume of plasma. Its low volume and its lower limit of quantification indicate that this approach is suitable for further characterisation of milrinone pharmacokinetics in both adults (inhalation) and neonates.

# Introduction

Cardiopulmonary bypass (CPB) is associated with a multitude of post-operative complications[1-4]. During CPB, in addition to blood/CPB apparatus interactions[5,6], lungs are minimally perfused and at weaning, may undergo ischemia-reperfusion injury that can eventually lead to pulmonary hypertension[7]. Compared to intravenous administration, inhaled milrinone has a more selective action on pulmonary vasculature with no systemic hypotension[8,9]. Results in patients given inhaled milrinone before cardiac surgery indicate plasma concentrations below 10 ng/mL shortly after weaning from cardiopulmonary bypass[10], and often under the lower limit of quantitation (LLOQ) 10 hours after inhalation (unpublished results). Thus, to fully characterize the elimination phase of inhaled milrinone, an improved sensitivity is required. Also revealed, due to the many concomitant medications given before, during and after CPB, chromatographic interferences were often observed at milrinone retention time, making quantification impossible. Therefore, a higher degree of specificity was also deemed necessary.

Milrinone, is also a possible treatment for several conditions in pediatric populations ranging from neonates to children. Milrinone is currently given intravenously to neonates in the case of persistent pulmonary hypertension of the newborn (PPHN). This life-threatening condition affects 2-6 infants in 1000[11] and 10% of all preterm and term neonates that require neonatal intensive care[12]. Use of intravenous milrinone has been shown to offer advantages in PPHN[13,14], when combined with inhaled nitrous oxide. Although inhaled milrinone could represent an interesting alternative, current pharmacokinetic data in neonates[15] remains quite limited.

Inhaled milrinone is also emerging as a treatment option for children with pediatric pulmonary hypertension due to acyanotic congenital heart disease which, when leading to right heart failure, is a leading cause of mortality and morbidity in cardiac patients[16]. Furthermore, milrinone was recently tested in the context of post-surgical support for neonates after cardiovascular surgery[17].

Milrinone now appears as a pediatric treatment option that would greatly benefit from pharmacokinetic characterization and dose optimization. For doing so, a low plasma volume analytical method and a higher degree of sensitivity are required. in particular for inhaled milrinone studies.

Few analytical methods for the determination of milrinone in human plasma have been published using HPLC-UV[10,18-20]. The LLOQ (based on 1 mL of plasma) reached with UV detection is approximately 1 ng/mL and, most importantly, lacked the high specificity required in cardiac patients. More recently, an HPLC-MS/MS assay[21] was proposed for therapeutic drug monitoring of milrinone given as an intravenous infusion in pre- or post-surgical cardiac patients. While the specificity issue was solved, the sensitivity reached (0.66 ng/mL) was not high enough, especially for its application in neonates. The aim of this report is to propose a validated HPLC-MS/MS micromethod for determination of milrinone plasma levels that achieves both a high sensitivity and selectivity.

# Methods and Materials

## Chemicals and Reagents

Milrinone was kindly supplied by Sandoz (Boucherville, QC, CAN). Olprinone hydrochloride, the internal standard (IS), was purchased from Toronto Research Chemicals (Toronto, ON, CAN). HPLC grade methanol, ethyl-acetate (Fisher Scientific, Nepean, ON, CAN) and ammonium acetate (Sigma Aldrich, St. Louis, MO, USA) were used.

## Standard Solutions and Buffers

Ammonium acetate 10 mM was prepared daily from a 2M stock solution. Stock solutions (1 mg/mL) of pure milrinone standard were prepared in methanol and stored at −20° C. Working solutions (10 000, 1000, 100 ng/mL) were prepared extemporaneously in water. A stock solution of the IS (10 000 ng/mL) was prepared in water, aliquoted and stored at −20° C. Working solutions (1000 ng/mL water) were prepared when needed.

## Calibration and Quality Control Samples

Twelve milrinone concentration standards, 0.3125 (LLOQ), 0.625, 1.25, 2.5, 5, 10, 20, 40, 80, 160, 320, 640 ng/mL and a blank plasma sample were used to establish plasma calibration curves. The samples were prepared extemporaneously by serial 1:1 dilutions using previously screened blank plasma. Quality control (QC) samples were prepared by spiking blank plasma with milrinone stock solutions to obtain final concentrations of 0.5 2, 50, 100 and 500 ng/mL. Calibration curves were fitted to a quadratic regression using a weighted least square regression ($1/y^2$ nominal) with the Agilent MassHunter Workstation Data Acquisition software (Version B.04.01, Agilent Technologies, Santa-Clara, CA, USA).

## Plasma Sample Preparation

In 1.5 mL conical polypropylene microtubes, 25 µL of IS was added to 50 µL of plasma. Subsequently, 500 µL of ethyl acetate was added, followed by horizontal agitation for 2 minutes (Eberbach, Ann Arbor, MI) and 4 min centrifugation. Then, 400 µL of the

supernatant were evaporated (Speedvac Plus model SC210A; Savant Instruments, Farmingdale, NY). Prior to HPLC-MS/MS analysis, samples were reconstituted in 25 µL of mobile phase and vortexed for 10 seconds. Samples were then transferred to vials and 5 µL injected.

## HPLC-MS/MS Analysis

### Chromatographic Conditions

The HPLC system was an Agilent 1200 (Agilent Technologies, Santa Clara, CA, USA) which consisted of an autosampler (Hi-ALS SL4), a column oven (TCC SL), a degasser and a binary pump (Bin pump SL). Separation of analytes was performed on a Zorbax Eclipse XDB-C18 (4.6 x 150 mm 5 µm) protected by a security cartridge system and using an isocratic mobile phase (methanol:10 mM ammonium acetate; 45:55 v/v) with a flow of 1 mL/min. Injection volume was 5 µL. Running time was of 2.6 min with overlap for flushing and syringe filling. The C-18 column was kept at 50°C.

### Mass Spectrometry Conditions

All analyses were performed on an Agilent 6460 triple quadrupole mass spectrometer equipped with an electrospray ion source (Agilent Technologies, Santa Clara, CA, USA). The ion source was operated in negative mode with nozzle voltage of -500 V. Compound-dependent parameters are listed in Table 1. Other mass spectrometer parameters were set as follows: the drying gas (nitrogen) temperature was 275°C with a flow of 5 L/min; the nebulizer pressure was 45 PSI; the sheath gas had a temperature of 325°C with a flow of 11 L/min. Capillary voltage was 3500 V. All data were acquired, analyzed and processed with the Agilent MassHunter Workstation Data Acquisition software. An example of milrinone and olprinone chromatogram extractions using their respective optimal transitions can be seen in Fig. 1. Retention times for milrinone and olprinone were 2.02 and 2.19, respectively.

### Assay Validation

Specificity was tested by obtaining blank drug-free plasma samples from healthy subjects and from patients immediately before inhalation of milrinone (pre-dose). Samples from healthy subjects and patients were assayed to determine whether

endogenous plasma components or anesthesia medication cause interference at the retention times of the analyte or IS. To this end, chromatograms of blank samples of both healthy subjects and patients were compared to milrinone and olprinone-containing samples. Blank plasma samples from four previously tested patients that exhibited interfering compounds under our previous assay conditions[10] were re-assayed. Interactions with the analyte or IS were ruled out. Linearity was assessed by 10 12-point calibration curves covering the expected clinical range (0.3125–640 ng/mL) on 10 separate days. Sensitivity was evaluated by observing the inter-day variability of 10 LLOQ calibration standards over 10 different days. Recovery was assessed by comparing the peak heights of milrinone spiked prior to and after extraction for six replicate sets of samples spiked at 2.5 and 100 ng/mL. Recovery of the IS was also determined by comparing the peak heights of four extracted samples with the 100% value determined using *in vitro* samples. Intra-assay precision and accuracy were assessed in plasma as follows: 0.5, 2, 50, 100 and 500 ng/mL QC concentrations were assayed in replicates of 6. All samples were assayed on the same day and their back-calculated concentrations determined from the calibration curve prepared the same day. Inter-assay precision and accuracy were assessed in the following way: 2, 100 and 500 ng/mL QC concentrations were assayed in duplicate for the first set of five calibration curves and 0.5 and 50 ng/ml QC concentrations were assayed in duplicate for the second set of five calibration curves. One curve with its respective QC samples was assayed per day for a total of 10 calibration curves over 10 different days. Precision was expressed as the coefficient of variation (C.V. %) and accuracy as the percent bias (%). Accuracy was determined by comparing the calculated concentration of the extracted milrinone plasma standard with the nominal concentration of milrinone.

**Stability**

Short-term stability (5h) was verified in four replicates of plasma standards at 2 and 500 ng/mL thawed at room temperature and kept at this temperature for 5h before analysis and comparison to fresh samples. Injector stability (24h) was verified in four replicates of plasma standards at 2 and 500 ng/mL injected in two different runs, 24 hours apart with two different calibration curves. To test freeze-thaw cycle stability, four calibration

standards were prepared and frozen at -70˚C for 24h. Those samples were then thawed at room temperature and analyzed alongside fresh samples of the same concentration. Samples were refrozen under the same conditions and freeze-thaw cycle repeated one more time before reanalysis. Stability of extracts (2 and 500 ng/mL), stored in the refrigerator or the autosampler for 24h was also tested. Results were compared with those obtained for the freshly prepared samples.

## Clinical Application to Cardiac Patients Undergoing Cardiopulmonary Bypass

This method was developed to determine milrinone plasma concentrations in cardiac patients after a 5 mg dose (Milrinone Lactate 1 mg/ml; Pharmaceutical Partners of Canada Inc., Richmond Hill, ON, CAN) inhaled over a 20 min period. Following ethics committee approval and permission from Health Canada, informed and written consent was obtained from all participating subjects. Blood samples were obtained before starting inhalation (time zero), during inhalation, and up to 10 hours after inhalation. Samples were kept in an ice-water bath for less than 10 min before centrifugation. Plasma was immediately flash-frozen on dry ice and stored at −70˚ C until analysis. Milrinone plasma concentration-time profile obtained in one patient is shown in Fig. 2.

# Results and Discussion

## Method optimization

The current method was developed taking advantage of the sensitivity and specificity provided by mass spectrometry. Compared to Nguyen et al.[10], several changes were made for method optimization. Required volume of plasma was reduced to 50 µL, optimizing the use of clinical samples and facilitating pharmacokinetic studies in children and neonates. In absence of a deuterated or C-13 milrinone IS, which may be considered suboptimal, the former IS (amrinone) was changed to olprinone due to subject-related variations that induced a systematic bias when comparing patient samples to calibration standards. Using olprinone as IS, within assay precision was acceptable (CV : 13.88 %, n = 21). Furthermore, to reduce the costs incurred for milrinone extraction, the SPE was changed to a liquid-liquid extraction. In preliminary analyses, liquid-liquid extraction using ethyl acetate/dichloromethane (4:1 v/v) showed no advantage. The mobile phase was simplified from ACN:THF-$NaH_2PO_4$ buffer pH 3 (gradient ranging from 10:90 to 40:60,) to methanol:10 mM ammonium acetate (isocratic 45:55 v/v). These changes result in a method that has, to our knowledge, the lowest LLOQ for plasma milrinone quantification: 0.3125 ng/mL. This LLOQ was necessary for quantifying milrinone plasma levels up to 10 hours post-dose.

## Assay Validation

Extracted MRM signals of milrinone and olprinone from a cardiac patient blank, a low calibration standard (0.3125 ng/mL, LLOQ), a medium calibration standard (80 ng/mL) and cardiac patient sample (610 min) are shown in Fig. 3. Both clinical and calibration samples were shown to be free of any interference with milrinone. Prior to the current method, milrinone specificity during CPB was a major drawback. When necessary during CPB, support medication had to be administered to patients, generating chromatographic interference during HPLC-UV analysis. No such difficulties were observed with the current method due to the benefits of mass spectrometry quantification. Indeed, patients from an ongoing study showing chromatographic interferences were successfully reanalyzed; an example is shown in Fig. 4.

The 10 milrinone calibration curves (0.3125 to 640 ng/mL) were fitted to quadratic regressions and showed a coefficient of determination ($r^2$) of 0.9954 ± 0.0024 (n = 10). The LLOQ showed mean inter-assay precision and accuracy bias of 5.97% and 0.65% (n = 10), respectively, indicating acceptable sensitivity. Mean recoveries of milrinone for the 2.5 and 100 ng/mL concentrations were 78.0 ± 2.1% and 78.9 ± 6.6 % (n = 6), respectively.

**Precision and Accuracy**

Intra and inter-assay precision and accuracy results can be found in Table 2. Pooled QCs and LLOQ over the 10 days showed that accuracy averaged 98.25% and ranged from 96.00 to 101.09 (n = 60). Intra- and inter-day precisions (CV%) were ≤ 3.02% and ≤ 11.42%, respectively.

**Stability**

Sample short-term stability (5h) showed accuracy ranging from 90 to 102%. Injector stability samples (24h) showed accuracy ranging from 92 to 106%. Freeze-thaw cycle stability samples showed accuracy ranging from 94 to 103% after two freeze-thaw cycles. For stability of extracts stored in the refrigerator or autosampler for 24h, no significant difference was observed when compared with values obtained from freshly prepared samples.

## Clinical Application

***Cardiac Patients Undergoing Cardiopulmonary Bypass.***

Milrinone plasma concentration-time profile obtained in one patient (Fig. 2) shows a maximum concentration of 40.63 ng/mL occurring 10 min after starting milrinone inhalation. Ten hours after inhalation, milrinone plasma levels were 0.33 ng/mL, just above the LLOQ. With the current method, full characterization of the terminal half-life was achieved.

***Persistent Pulmonary Hypertension of the Newborn***

The pharmacokinetic study of McNamara et al[15] used an assay with a LLOQ of 10 ng/mL and using 100 µL of plasma allowing six blood samples of 800 µL per neonate.

The current method, with a LLOQ of 0.3125 ng/mL and using 50 µL of plasma would, by allowing additional samples, a thorough characterization of milrinone overall pharmacokinetics. In addition, it would also allow for the determination of single dose pharmacokinetics and further exploration of milrinone use as a treatment option for PPHN.

***Acyanotic congenital heart disease and newborns undergoing cardiovascular surgery***

Currently, no studies have characterized inhaled milrinone pharmacokinetics in a pediatric population. Singh et al. explored the efficacy of milrinone in this particular condition and showed a 14.9% decrease in mean pulmonary arterial pressures. Such pharmacodynamic studies would greatly benefit from concentration-response profiles if one wished to optimize drug administration. In the case of post-operative support, intravenous milrinone was shown to be beneficial[17]. Inhaled milrinone has yet to be tested for this indication and would be a possible research avenue. However, the sensitivity of Pellicer et al.'s assay (LLOQ: 2 ng/ml) would not allow adequate characterization of inhaled milrinone terminal half-life. Even more than for adults, plasma exposure following milrinone inhalation in pediatric and/or neonatal studies is expected to be fairly low. Therefore, the current HPLC-MSMS method offers the required sensitivity and specificity to provide adequate characterization of inhaled milrinone pharmacokinetics in this population.

## Conclusions

A highly sensitive and specific HPLC-MS/MS assay for inhaled milrinone was developed that utilizes a low-cost rapid extraction procedure for a low sample volume (50 µL), is milrinone-specific in a cardiac surgery context and has good sensitivity (LLOQ: 0.3125 ng/mL). This validated assay allows adequate determination of milrinone plasma levels after its administration by inhalation in patients undergoing CPB surgery and fulfills the need for a low volume and sensitive method in pediatric and even neonate pharmacokinetic studies.


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# References


1 Denault, A., Deschamps, A., Tardif, J. C., Lambert, J. & Perrault, L. Pulmonary hypertension in cardiac surgery. *Curr Cardiol Rev. 2010;* 6: 1-14,

2 Gorcsan, J. *et al.* Assessment of the immediate effects of cardiopulmonary bypass on left ventricular performance by on-line pressure-area relations. *Circulation*. 1994; 89: 180-190

3 Hind, C. R. *et al.* Effect of cardiopulmonary bypass on circulating concentrations of leucocyte elastase and free radical activity. *Cardiovasc Res.* 1988*;* 22: 37-41

4 Gnanadurai, T. V., Branthwaite, M. A., Colbeck, J. F. & Welman, E. Lysosomal enzyme release during cardiopulmonary bypass. *Anaesthesia*. 1977; 32: 743-748

5 Courtney, J. M., Sundaram, S., Matata, B. M., Gaylor, J. D. S. & Forbes, C. D. Biomaterials in cardiopulmonary bypass. *Perfusion*. 1994; 9: 3-10

6 Royston, D. The inflammatory response and extracorporeal circulation. *J Cardiothorac Vasc Anesth.* 1997; 11: 341-354

7 Urdaneta, F. *et al.* Treating pulmonary hypertension post cardiopulmonary bypass in pigs: milrinone vs. sildenafil analog. *Perfusion.* 23: 117-125, doi:10.1177/0267659108094739 (2008).

8 Wang, H., Gong, M., Zhou, B. & Dai, A. Comparison of inhaled and intravenous milrinone in patients with pulmonary hypertension undergoing mitral valve surgery. *Adv Ther.* 2009; 26: 462-468

9 Sablotzky, A., Czeslick, E. G., Scheubel, R. & Grond, S. Selective pulmonary vasodilation with inhaled aerosolized milrinone in heart transplant candidates. *Can J Anaesth*. 2005; 52:1076-1082

10 Nguyen, A. Q. N., Théorêt, Y., Chen, C., Denault, A. & Varin, F. High performance liquid chromatography using UV detection for the quantification of milrinone in plasma: improved sensitivity for inhalation. *J Chromatogr B Analyt Technol Biomed Life Sc.* 2009; 877: 657-660

11 Walsh-Sukys, M. C. *et al.* Persistent pulmonary hypertension of the newborn in the era before nitric oxide: practice variation and outcomes. *Pediatrics*. 2000; 105, 14-20

12 Steinhorn, R. H. Pharmacotherapy for pulmonary hypertension. *Pediatr Clin North Am. 2012;* 59: 1129-1146

13 McNamara, P. J., Laique, F., Muang-In, S. & Whyte, H. E. Milrinone improves oxygenation in neonates with severe persistent pulmonary hypertension of the newborn. *J Cri Care*. 2006; 21: 217-222

14 Bassler, D., Choong, K., McNamara, P. & Kirpalani, H. Neonatal persistent pulmonary hypertension treated with milrinone: four case reports. *Biol Neonate*. 2006; 89: 1-5

15 McNamara, P. J., Shivananda, S. P., Sahni, M., Freeman, D. & Taddio, A. Pharmacology of milrinone in neonates with persistent pulmonary hypertension of the newborn and suboptimal response to inhaled nitric oxide. *Pediatr Crit Care Med.* 2013; 14: 74-84

16 Singh, R. *et al.* Inhaled nitroglycerin versus inhaled milrinone in children with congenital heart disease suffering from pulmonary artery hypertension. *J Cardiothorac Vasc Anesth*. 2010; 24: 797-801

17 Pellicer, A. *et al.* Phase 1 study of two inodilators in neonates undergoing cardiovascular surgery. *Pediatr Res*. 2013; 73, 95-103

18 Edelson, J., Koss, R. F., Baker, J. F. & Park, G. B. High-performance liquid chromatographic analysis of milrinone in plasma and urine. Intravenous pharmacokinetics in the dog. *J Chromatogr*. 1983; 276, 456-462

19 Oddie, C. J., Jackman, G. P. & Bobik, A. Analysis of milrinone in plasma using solid-phase extraction and high-performance liquid chromatography. *J Chromatogr*. 1986; 374, 209-214

20 Brocks, D. R., Spencer, T. J. & Shayeganpour, A. A sensitive and specific high performance liquid chromatographic assay for milrinone in rat and human plasma using a commercially available internal standard and low sample volume. *J Pharm Pharm Sci.* 2005; 8, 124-131

21 Chihoho, B. *et al.* A clinical assay for the measurement of milrinone in plasma by HPLC mass spectrometry. *Biomed Chromatogr*. 2012; 26, 566-570

# Tables

**Table 1.** MRM transitions and fragmentation parameters used for the quantification of milrinone using olprinone as the internal standard.

| **Compound name** | MRM m/z transition | Dwell | Fragmentor (V) | Collision energy (eV) | Polarity |
|---|---|---|---|---|---|
| **Olprinone** | 252.1 → 156.1 | 200 | 120 | 36 | Positive |
| **Milrinone** | 212.1 → 140.0 | 200 | 115 | 32 | Positive |

**Table 2.** Intra-assay and inter-assay precision and accuracy.

| | | Concentration (ng/mL) | | Precision | Accuracy |
|---|---|---|---|---|---|
| | **n** | **Nominal** | **Measured mean ± SD** | **Coefficient of variation (%)** | **Bias (%)** |
| **Intra-assay** | | | | | |
| | 6 | 0.5 | 0.51 ± 0.02 | 4.33 | 1.45 |
| | 6 | 2 | 1.92 ± 0.10 | 4.96 | -2.60 |
| | 6 | 50 | 48.49 ± 3.57 | 7.36 | -3.02 |
| | 6 | 500 | 504.21 ± 12.22 | 2.42 | 0.84 |
| **Inter-assay** | | | | | |
| | 10 | 0.3125 | 0.32 ± 0.02 | 5.97 | 0.65 |
| | 10 | 0.5 | 0.48 ± 0.03 | 6.12 | -4.00 |
| | 10 | 2 | 1.95 ± 0.08 | 3.94 | -2.68 |
| | 10 | 50 | 48.11 ± 3.57 | 5.21 | -3.78 |
| | 10 | 100 | 98.24 ±11.22 | 11.42 | -1.76 |
| | 10 | 500 | 505.43 ± 6.64 | 1.71 | 1.09 |

# Figures

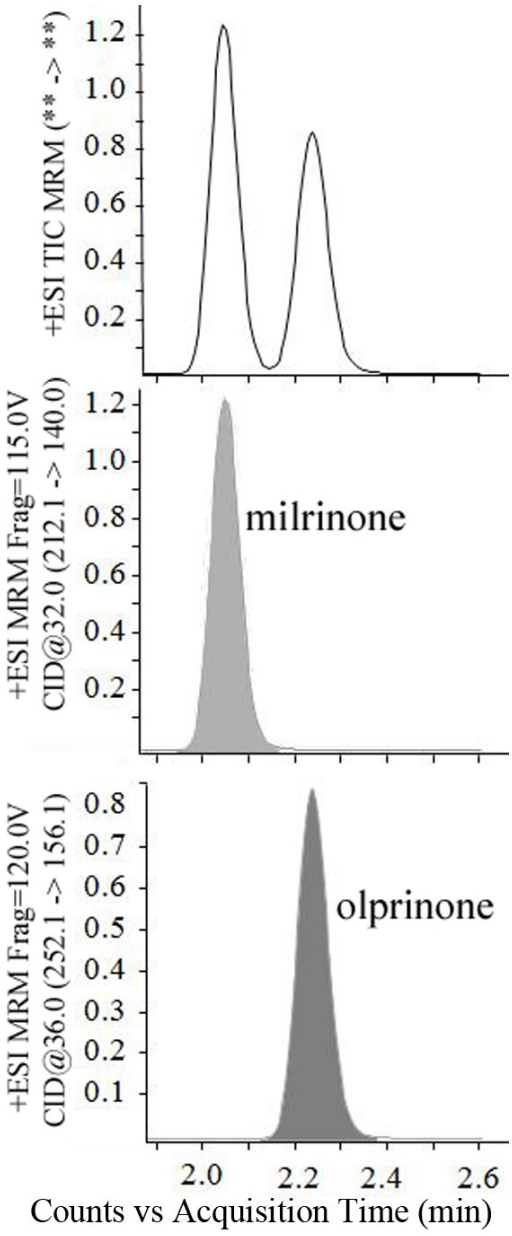


**Figure 1.** Initial chromatogram and extracted MRM signals of milrinone and olprinone from an extracted QC sample (100 ng/mL).

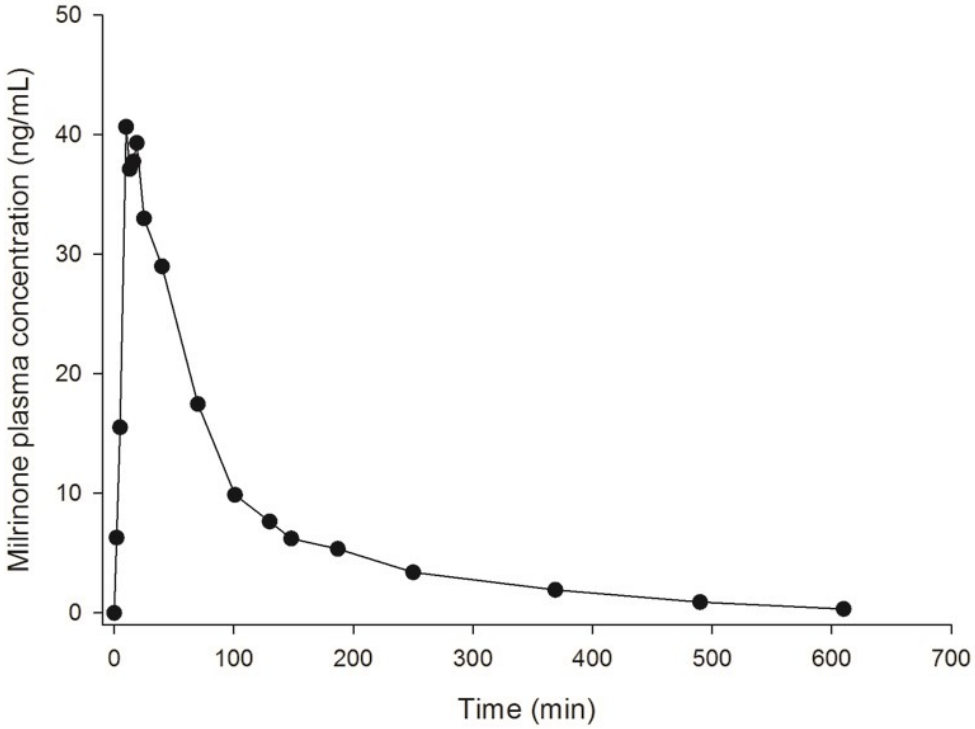


**Figure 2.** Milrinone concentration-time profile in a patient having received a dose of 5 mg of milrinone by inhalation over 20 min.

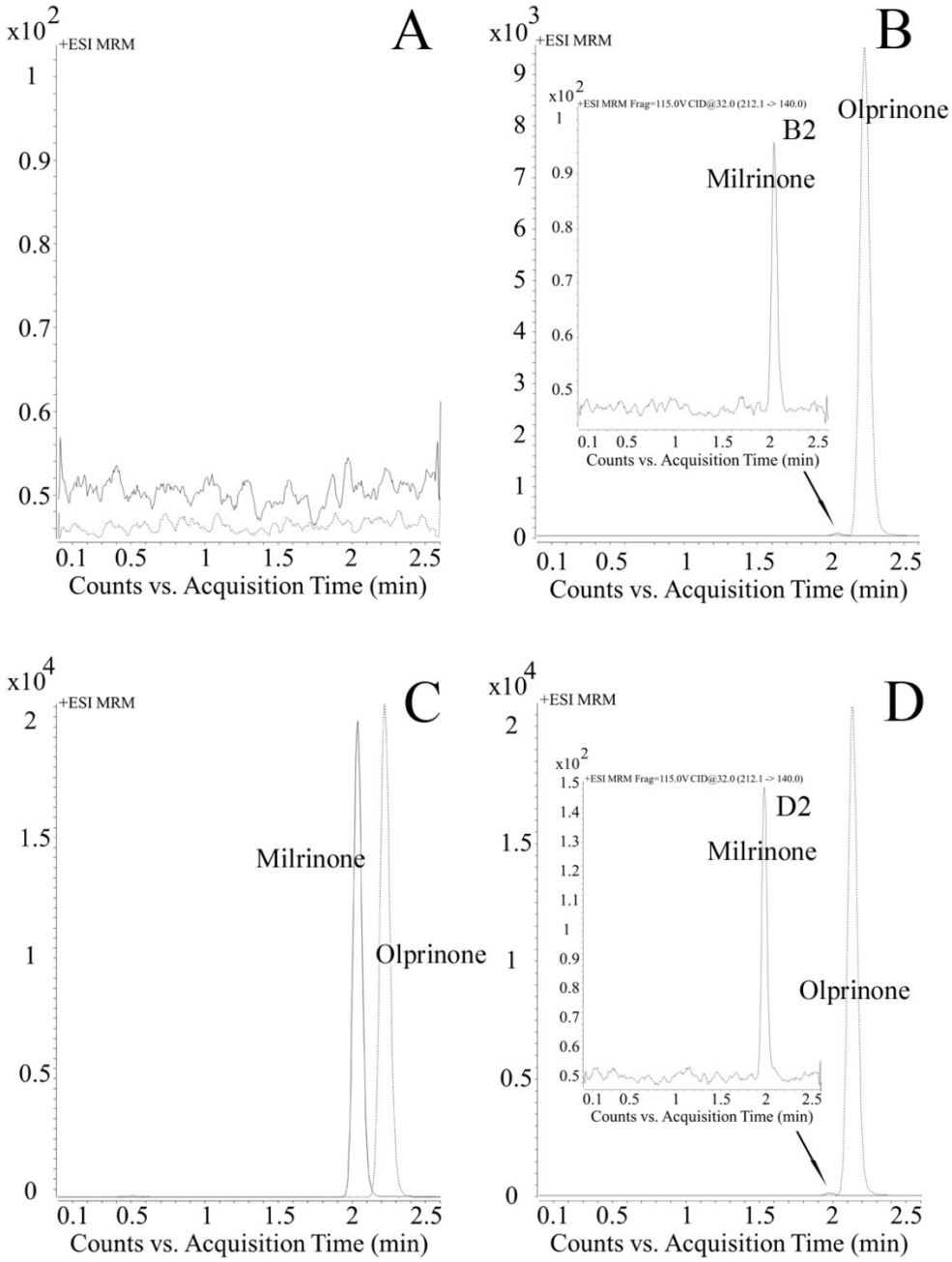


**Figure 3.** Overlaid extracted milrinone (212.1 -> 140.0) and olprinone (252.1 -> 156.1) MRM signals from (A) a blank cardiac patient plasma sample (B), a plasma calibration standard sample, LLOQ (0.3125 ng/mL), (C) a plasma calibration standard sample (80 ng/mL) and (D) a cardiac patient plasma sample 610 minutes after an inhalation of 5 mg of milrinone dose over 20 minutes (0.3344 ng/mL). B2 and D2 inserts are the magnified extracted milrinone (212.1 -> 140.0) MRM signal of the respective samples.

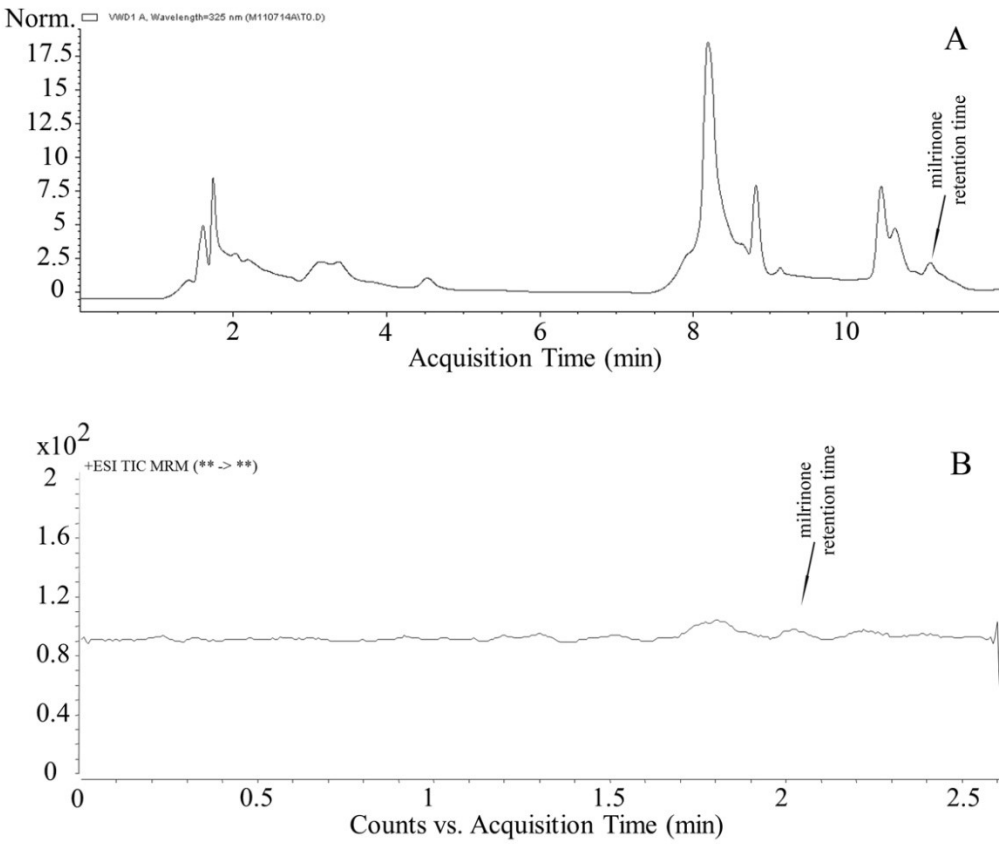


**Figure 4.** Comparison of bioanalysis method specificity. A blank plasma sample from the same patient was analyzed (A), using the method of Nguyen et al.[10] and (B) using the current method.

# ANNEXE III. Manuscrit n°6 (co-premier auteur): High-performance liquid chromatography assay using ultraviolet detection for urinary quantification of milrinone concentrations in cardiac surgery patients undergoing cardiopulmonary bypass

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**Short title:** HPLC method for urinary milrinone

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# Abstract

An analytical assay using liquid-liquid extraction and high-performance liquid chromatography with ultraviolet detection was developed for the quantification of total (conjugated and unconjugated) urinary concentrations of milrinone after the inhalation of a 5 mg dose in 15 cardiac patients undergoing cardiopulmonary bypass (CPB). Urine samples (700 µl) were extracted with ethyl-acetate and subsequently underwent acid back-extraction before and after deconjugation by mild acid hydrolysis. Milrinone was separated on a strong cation exchange analytical column. The mobile phase consisted of a constant mixture of acetonitrile: tetrahydrofurane-$NaH_2PO_4$ buffer (40:60 v/v, pH 3.0). Thirteen calibration curves were linear in the concentration range of 31.25-4000 ng/ml, using olprinone as the internal standard ($r^2$ range: 0.9911-0.9999, n = 13). Mean milrinone recovery and accuracy were respectively 85.2 ± 3.1% and ≥ 93%. Intra- and inter-day precisions (coefficients of variation, %) were ≤ 5% and ≤ 8%, respectively. Over a 24 h collection period, the cumulative urinary milrinone recovered from 15 patients was 26.1±7.7% of the nominal 5 mg dose administered. The relative amount of milrinone glucuronic acid conjugate was negligible in the urine of patients undergoing CPB This method proved to be reliable, specific and accurate to determine the cumulative amount of total milrinone recovered in urine after inhalation.



**Keywords:** HPLC; Inhalation; Milrinone; Pharmacokinetics; Urine.


**Abbreviations:** CPB, cardiopulmonary bypass; CV, coefficient of variation; LLOQ, lower limit of quantification; QC, quality control; SPE, solid phase extraction.

# Introduction

Milrinone is a phosphodiesterase 3 inhibitor commonly used for its vasodilator effect in patients undergoing cardiac surgery to facilitate weaning from cardiopulmonary bypass (CPB) (Denault et al, 2006; Haddad et al, 2009). The pulmonary reperfusion syndrome often occurs during and after weaning from CPB and would result in pulmonary hypertension and right heart complications (Shafique et al, 1993). Intravenous milrinone is generally used as a treatment following CPB but its main drawback is the presence of systemic hypotension. Inhalation of milrinone using a nebulizer before CPB has been suggested as an alternative route of administration to reduce pulmonary pressure after CPB while avoiding systemic hypotension (Denault et al, 2006). Preliminary results from our laboratory indicate that the estimated delivered dose and systemic exposure after inhaled milrinone may vary up to twofold according to the type of nebulizer used (Nguyen et al, 2010). However, the cumulative amount of milrinone recovered in urine after inhalation in cardiac surgical patients undergoing CPB has not been characterized.

In healthy subjects, milrinone has been reported to be mainly excreted in the urine in two forms: unchanged (83%) and as an O-glucuronic acid conjugate (12%) (Primacor®). Alousi et al (1985), reported a similar mean extent of glucuronidation in dogs using radioactive compounds and separation by HPLC (15 %). Therefore, determination of the total amount of milrinone excreted in urine after nebulization would allow us to assess the systemic bioavailability after this highly variable route of administration. To the best of our knowledge, only three analytical methods have been published for the determination of milrinone in urine for studies in dogs (Edelson et al, 1983) and humans (Baranowska et al, 2011; Magiera et al, 2012). However, most of the reported assays have not been fully validated, were not reproducible under similar conditions, and used an internal standard that was commercially unavailable (Edelson et al, 1983). No validated method has been reported for the determination of milrinone in human urine. The scope of this paper is to provide a validated high performance liquid chromatography (HPLC) assay using ultraviolet (UV) detection for the quantification of milrinone total urinary concentration after inhalation and determine the relative amount of its O-glucuronic acid conjugate form in cardiac surgical patients undergoing CPB.

# Experimental

## Chemicals and reagents

Milrinone standard (99.2 % pure) was kindly provided by Sandoz (Boucherville, QC, CAN). The internal standard, olprinone, was purchased from Toronto Research Chemicals (Toronto, Ont, CAN). An O-glucuronic acid reference standard for milrinone was not available. Chemical structures are illustrated in Fig. 1. All solvents and reagents used were of HPLC grade. The mobile phase was filtered through a 0.22 µm type HVLP membrane before use (Millipore, Billerica, MA, USA). Ultrahigh purified water was obtained from Milli-Q water dispensing system by Millipore Corporation (Billerica, MA, USA).

## Chromatographic conditions

The HPLC analysis was performed on an Agilent HP1200 series HPLC system (Agilent Technologies, Santa-Clara, CA, USA) equipped with a multiple solvent delivery system and a variable wavelength UV/visible detector. Chromatographic separations were carried on a Waters Spherisorb strong cation exchange column (150 mm X 4.6 mm I.D., 5 µm HiChrom Ltd., Reading, Berkshire, UK) protected by a security filter system. The mobile phase consisted of a mixture of acetonitrile and tetrahydrofurane-$NaH_2PO_4$ buffer (40:60 v/v) delivered at 1.6 ml/min with a run time of 5.5 min. Tetrahydrofurane was added to 0.05M $NaH_2PO_4$ buffer (pH 3.0) immediately before HPLC analysis to obtain the final tetrahydrofurane-$NaH_2PO_4$ buffer (5:218, v/v). The chromatography was performed at ambient temperature. Injection volume was 120 µl and UV detection at 325 nm. Chromatographic peaks were integrated by the ChemStation software (Agilent Technologies, Santa-Clara, CA, USA).

## Stock and working standard solutions

Stock solutions (1 mg/ml) of milrinone were prepared in methanol and stored at -20˚C. A stock solution of olprinone (1 mg/ml) was aliquoted in water and stored at -20˚C. Working solutions of both stock solutions were prepared in water. The phosphate buffer used for sample preparation was adjusted to pH 7.4 by mixing stock 0.5M $KH_2PO4$ and

0.5M $Na_2HPO_4$ solutions. The neutralizing solution used for ending incubation and neutralising after the acid back-extraction was prepared by adding 0.6 ml 10N NaOH to 9.4 ml of phosphate buffer (0.5M, pH 7.0).

## Calibration and quality control samples

Eight milrinone calibration standards, 31.25 (lower limit of quantification, LLOQ), 62.5, 125, 250, 500, 1000, 2000 and 4000 ng/ml were used to establish calibration curves in urine. Calibration samples were prepared by serial 1:1 dilutions with previously screened blank healthy subject urine. Calibration curves were generated by plotting the analyte/internal standard peak-height ratio against nominal milrinone concentration. Quality control (QC) samples were prepared by spiking blank healthy subject urine with milrinone stock solutions to obtain final concentrations of 160 and 1600 ng/ml. Prior to extracted-sample analysis, milrinone and internal standard daily retention times were assessed by injecting *in vitro* samples containing each standard.

## Urine samples preparation

### Acid deglucuronidation

Since no O-glucuronic acid conjugate was available for milrinone, we established the optimal conditions for hydrolysis using urine samples collected from several end-stage renal failure patients having received inhaled milrinone before CPB. These patients were expected to have higher levels of the conjugate and acted as positive controls. We compared the results obtained at 65°C after various incubation times (up to 24 h) under mild acid conditions (pH ≈ 1.5) with those obtained using *Helix pomatia* β-glucuronidase (Sigma Chemicals, St-Louis, Mo, USA) enzymatic hydrolysis. A duration of 24 h for mild acid hydrolysis yielded the same results as the optimal enzymatic hydrolysis conditions (5,000 Units, 6 h, 45°C). Stability of both milrinone and olprinone under these mild acid hydrolysis conditions was then verified. ANOVA analysis showed that the effects of temperature and/or acidity were not statistically significant.

Urine samples (700 µl) obtained from patients treated with milrinone (please refer to clinical application) and calibration standards were added 100 µl of internal standard and 200 µl of HCL 0.5N before incubation at 65˚C for 24 hours. Incubation was ended by

placing samples on ice and transferring 600 µl in a glass vial containing 50 µl of neutralising solution. For non-incubated samples, the 0.5N HCL and neutralising solutions were replaced with purified water for a similar preparation of samples prior to extraction.

**Extraction**

Following incubation 500 µl of sample was pipetted into 250 µl of phosphate buffer, 100 µl of internal standard and 5 ml of ethyl acetate. The samples were then shaken for 15 min, centrifuged (*IEC Centra-8R* centrifuge) at 3200 rpm (1280 g) for 15 min and placed at -70˚C for 20 min. The ethyl acetate layer was transferred in a clean 15 ml conical tube containing 400 µl of HCl 0.1N. The samples were vortexed for 1 min, centrifuged (*IEC Centra-8R* centrifuge) for 10 min at 3200 rpm (1280 g) and the ethyl acetate was subsequently discarded. Thus 25 µl of neutralising solution were added to 300 µl of the acidified phase and 120 µl was injected in the HPLC.

## Method validation

The analytical method was validated for selectivity, linearity, sensitivity, extraction recovery, precision, accuracy, matrix effects and stability according to US Food and Drug Administration (FDA) guidance for bioanalytical method validation (2001).

**Selectivity**

Blank drug-free urine samples were obtained from two healthy subjects and 28 urine samples were obtained immediately before inhalation of milrinone (pre-dose) in patients undergoing cardiac surgery. Matrix effects for analytes were evaluated through comparison of samples from direct injections of standard solutions, known-concentration spiked healthy subject samples and patient samples. Samples were assayed to determine whether endogenous urine components or anesthesia medication cause interferences at the retention times of the analyte or internal standard. To this end, chromatograms of blank samples (healthy subjects and patients) were compared to milrinone and olprinone-containing samples.

**Linearity**

Eight calibration standards (31.25 – 4000 ng/ml) covering the expected clinical range were prepared in urine. Linearity was assessed using a weighted least square linear regression ($1/x^2$ nominal).

**Sensitivity**

Milrinone was spiked at a concentration of 31.25 ng/ml (LLOQ) in healthy subject urine and was extracted and injected on 8 different days (inter-day).

**Recovery**

Four replicate sets of samples spiked at 60, 160 and 1600 ng/ml were prepared. Recovery was assessed by comparing the peak height ratio of milrinone spiked prior and after extraction. Recovery of the internal standard was also determined by comparing the peak heights of four extracted samples with the internal standard spiked prior and after extraction.

**Precision and accuracy**

The intra-assay precision and accuracy were assessed in urine as follows: 60, 160 and 1600 ng/ml QC concentrations were assayed in replicates of four. All samples were assayed on the same day and their back-calculated concentration determined from a calibration curve prepared the same day. Inter-assay precision and accuracy were assessed as follows: 60, 160 and 1600 ng/ml QC concentrations were assayed in duplicate for each calibration curve. One curve with its respective QC samples was assayed per day for a total of 13 calibration curves over 13 different days. Precision was expressed as the coefficient of variation (CV, %) and accuracy as the percent bias (%). Accuracy was determined by comparing the calculated concentration of the extracted milrinone urine standard with the nominal concentration of milrinone.

**Stability**

Short-term bench stability was verified in four replicates of urine standards (160 and 1600 ng/ml) thawed at room temperature for 24h before analysis. For the freeze-thaw cycle stability studies, four aliquots (160 and 1600 ng/ml) were prepared and frozen at -

70˚C for 24h. The samples were thawed unassisted at room temperature and analyzed alongside fresh samples of the same concentration. The samples were refrozen for 24h under the same conditions and the freeze-thaw cycle was repeated one more time before reanalysis. Processed samples stability was also tested for extracts (160 and 1600 ng/ml) stored in the refrigerator for 24h and remained in the autosampler for 24h. Results were compared with those obtained for the freshly prepared samples.

## Clinical application

Following permission from Health Canada (clinical trial application) and approval by the local ethics committee, informed consent was obtained from all participating subjects. As part of the protocol, milrinone concentrations in urine were determined for 15 patients having inhaled 5 mg of the study drug prior to initiation of CPB and cardiac surgery. Urine was collected over different intervals (n = 4-7), before drug administration, before CPB, post CPB, post-surgery and for a period of 24 hours post-nebulization and samples were stored at -70˚C until analysis. Pre-dose urine samples were verified to be free of endogenous or drug interferences under the anesthetic and surgical procedure. In order to determine the relative amount of milrinone's O-glucuronic acid conjugate form in cardiac surgical patients undergoing CPB, the amount of milrinone (total and free) was quantified in urine collected after milrinone administration by analysing all samples, once with and once without incubation, alongside their respective and similarly treated standard curves. Systemic bioavailability after inhaled milrinone was estimated by comparing the dose recovered in urine after 24 hours with the nominal dose (5 mg) initially administered to the patient.

# Results and Discussion

## Method optimization

The bioavailability and pharmacokinetics of inhaled milrinone have never been described but are expected to be highly variable due to the inherent losses of the drug delivery method. In pharmacokinetic studies involving inhaled drugs, due to these losses, an estimation of the percentage of the nominal dose actually being delivered to patients is required. In the case of milrinone, the molecule being excreted mostly unchanged or conjugated, measurement of total urinary excretion allows for a realistic approximation of the delivered dose. The purpose of this paper was to provide a low-cost, rapid and validated method for the quantification of total amount of milrinone in human urine.

A few analytical methods were available for determination of milrinone in urine. Recently, Magiera et al (Magiera et al, 2012) and Baranowska (Baranowska et al, 2011) described metamethods using solid-phase extraction (SPE) followed by UHPLC-UV and liquid-liquid extraction followed by UHPLC–ESI-MS/MS quantification, respectively. These methods required highly specialized and expensive equipment that offered a sensitivity level considered unnecessary to determine milrinone urine levels after commonly administered dose (50-80 μg/kg). Furthermore, being metamethods, they are not focused on the milrinone determination, resulting in longer running times. The HPLC-UV method described by Edelson et al (Edelson et al, 1983) for their pharmacokinetic study consisted of a liquid-liquid extraction followed by an acid back-extraction and separation on a $C_{18}$ analytical column. The latter method was used as a starting point for our method optimization. A major drawback of the Edelson et al (Edelson et al, 1983) assay was their internal standard (1,6-dihydro-2-ethyl-6-oxo-(3,4'-bypiridine)-5-carbonitrile) that, to our knowledge, is not commercially available.

Several potential candidates for our internal standard were screened: carbamazepine, amrinone, propranolol and finally olprinone. The mobile phase composition that allowed adequate separation of carbamazepine from the solvent front drastically reduced milrinone sensitivity. During validation procedures, patient samples spiked with

amrinone showed a systematic bias when compared to calibration standards prepared in subjects' blank urine. Although propranolol recovery was 63.5 ± 1.8 %, its absorbance signal at 325 nm was found too low compared to that of milrinone (optimal propranolol concentration for acceptable signal was 100 000 ng/ml of diluted urine). Olprinone had no interferences and showed consistency in absorbance signal at 325 nm between subjects and patients. Thus, with its sufficient recovery, olprinone was considered the most reliable and suitable internal standard. As the urinary concentrations of milrinone were expected to be relatively high after a dose of 5 mg, sensitivity was not deemed an issue and the volume of sample used for extraction was reduced to use less ethyl acetate and facilitate manipulation. In order to shorten the analysis time, our extraction method does not concentrate the sample, which resulted in a slightly lower recovery of milrinone but saved an hour of manipulation time while reducing baseline signal noise. When compared to Edelson et al (Edelson et al, 1983), our assay is fully validated using a commercially available internal standard, presents a shorter run time (5.5 versus 8 min), a slightly lower recovery (85 % vs 90 %), and an increased sensitivity (LLOQ = 31.25 vs 50 ng/ml).

## Assay validation

### Selectivity

Chromatograms of a healthy subject blank sample, a cardiac patient blank, a healthy subject blank spiked at 31.25 ng/ml (calibration standard, LLOQ) and a cardiac patient sample are shown in Fig. 2. The blank healthy subject urine used for each calibration curve (not necessarily in the fasted state) and urine sampled from each patient undergoing cardiac surgery (prior to milrinone administration) were analyzed and shown to be free of any interference. No matrix effects for milrinone and olprinone were observed. Variations in day-to-day elution times were the following: between 2.4 and 2.7 min for milrinone and between 4.4 and 4.6 min for the internal standard.

### Linearity

Calibration curves of milrinone in urine were linear from 31.25 to 4000 ng/ml with a mean coefficient of determination ($r^2$) of 0.9964 (n = 8; range: 0.9911-0.9999).

### Sensitivity

Mean inter-assay precision and accuracy for the LLOQ (31.25 ng/ml) were 2.3 % and 99 %, respectively (n = 13). The LLOQ's signal amplitude was found to be more than 10 times that of the baseline noise.

### Recovery

Mean recoveries of milrinone for the 60, 160 and 1600 ng/ml concentrations were 86.3, 84.1 and 84.8 % (n = 4), respectively. The mean recovery of the internal standard was 63.5 % (n = 4).

### Precision and accuracy

Intra and inter-assay precision and accuracy results are summarized in Table 1. Intra-assay precision study revealed a CV < 5 % (n = 4) for milrinone concentrations and accuracy ranged from 94 to 99 %. Inter-assay precision study revealed a CV < 8 % (n = 16) for milrinone concentrations and accuracy ranged from 92 to 100 %.

### Stability

For the short-term stability, accuracy ranged from 97 to 102 %. Accuracy ranged from 93 to 102 % after two freeze-thaw cycles. For the processed samples stability, accuracy following storage of extracts in the refrigerator and in the autosampler varied between 100 and 103 %.

## Clinical application

### Deglucuronidation

A mean cumulative amount of 1.103 mg ± 0.426 and 1.099 mg ± 0.403 were determined for free and total urinary milrinone, respectively. Individual percentages of glucuronic acid conjugate varied between - 8.9 % and 19 % (mean: - 0.6%) of the cumulative total amount measured. These results indicate that the amount of milrinone O-glucuronic acid conjugate in urine was negligible in our cardiac patients A mean extent of 12 % for O-glucuronidation has been reported in healthy volunteers (Primacor®). Alousi et al (Alousi et al, 1985) reported using a mild acid hydrolysis without providing a

description of the incubation conditions nor of the between subject variability in the content of the glucuronic acid conjugate.

In some of our patients, negative deconjugation results were obtained which, in our opinion, could be explained by the experimental error of our analytical assay. Furthermore, it has been reported that lysosomal enzyme release occurs during cardiopulmonary bypass in man, resulting in an increase of 35% in the activity of β-glucuronidase (Gnanadurai et al, 1977). This release of lysosomal enzymes originates from damaged renal cells (Hashimoto et al, 1993). This factor may also have contributed in reducing the O-glucuronic acid conjugate fraction in our cardiac patients undergoing CPB.

**Urinary pharmacokinetics**

In our 15 patients**,** the mean cumulative amount of milrinone recovered in urine was 26.1 ± 7.7% of the nominal 5 mg dose administered. The urinary pharmacokinetic profiles of free and total (free and conjugated) milrinone obtained from a patient having received 5 mg of milrinone inhaled over 20 min is shown in Fig. 3. In this patient, the free and total amount of milrinone recovered in urine over the 24 hour period were 1.128 and 1.238 mg, respectively; corresponding to 24.8 % of the initially nebulized dose (5 mg). In a previous *in vitro* study (Nguyen et al, 2009), our group tested the performance of the mesh nebulizer and quantified the overall loss of drug in the apparatus. In this study, the mean amount of milrinone delivered at the end of the endotracheal tube was estimated at 40 % of the initially nebulized dose (5mg). Although estimation of overall loss is not feasible *in vivo*, these results are compatible with the low percentage of milrinone recovered (relative to nominal dose) from the urine in our patients. Combined urinary and plasma pharmacokinetics are essential to shed light on this highly variable route of administration. In future pharmacokinetic studies, this method will prove helpful to assess the absolute or relative bioavailability after milrinone inhalation.

## Conclusion

A highly selective, rapid and low-cost HPLC assay with UV detection has been optimized and validated for the determination of total amount of milrinone in human urine. The method is simple and uses a commercially available internal standard. It was successfully applied to determine the systemic bioavailability and urinary pharmacokinetic profile of milrinone after inhalation in cardiac surgical patients undergoing CPB.

## Acknowledgements

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# References


Alousi AA, Fabian JR, Baker FJ, Stroshane MR. Milrinone. New Drugs Annual: Cardiovascular Drugs. 1985;3:39.

Baranowska I, Magiera S, Baranowski J. UHPLC method for the simultaneous determination of beta-blockers, isoflavones, and flavonoids in human urine. Journal of chromatographic science. 2011 Nov-Dec;49(10):764-73.

Denault AY, Lamarche Y, Couture P, Haddad F, Lambert J, Tardif JC, et al. Inhaled milrinone: a new alternative in cardiac surgery? Seminars in cardiothoracic and vascular anesthesia. 2006 Dec;10(4):346-60. 10.1177/1089253206294400

Edelson J, Koss RF, Baker JF, Park GB. High-performance liquid chromatographic analysis of milrinone in plasma and urine. Intravenous pharmacokinetics in the dog. Journal of chromatography. 1983 Sep 9;276(2):456-62.

Gnanadurai TV, Branthwaite MA, Colbeck JF, Welman E. Lysosomal enzyme release during cardiopulmonary bypass. Anaesthesia. 1977 Sep;32(8):743-8.

Haddad F, Couture P, Tousignant C, Denault AY. The right ventricle in cardiac surgery, a perioperative perspective: II. Pathophysiology, clinical importance, and management. Anesthesia and analgesia. 2009 Feb;108(2):422-33. 10.1213/ane.0b013e31818d8b92

Hashimoto K, Nomura K, Nakano M, Sasaki T, Kurosawa H. Pharmacological intervention for renal protection during cardiopulmonary bypass. Heart and vessels. 1993;8(4):203-10

Magiera S, Baranowska I, Kusa J. Development and validation of UHPLC-ESI-MS/MS method for the determination of selected cardiovascular drugs, polyphenols and their metabolites in human urine. Talanta. 2012 Jan 30;89:47-56. 10.1016/j.talanta.2011.11.055

Nguyen AQN, Denault AY, Perrault LP, Varin F. Exploratory PK/PD study after inhaled milrinone in cardiac patients. Canadian Journal of Anesthesia/Journal canadien d'anesthésie. 2010;57(S1):19. 10.1007/s12630-010-9415-0

Nguyen AQN, Denault AY, Perrault LP, Varin F, editors. In vivo-in vitro correlation between early systemic exposure and delivered dose of inhaled milrinone using two types of nebulizers. American Conference on Pharmacometrics; 2009 2009. The AAPS Journal: The AAPS Journal; 2009.

Nguyen AQN, Theoret Y, Chen C, Denault AY, Varin F. High performance liquid chromatography using UV detection for the quantification of milrinone in plasma: improved sensitivity for inhalation. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2009 Mar 1;877(7):657-60. 10.1016/j.jchromb.2009.01.024

Primacor® Product Information [Internet]. Primacor® [cited 2013 nov 07]. Available from: http://products.sanofi.com.au/aus_pi_primacor.pdf

Shafique T, Johnson RG, Dai HB, Weintraub RM, Sellke FW. Altered pulmonary microvascular reactivity after total cardiopulmonary bypass. The Journal of thoracic and cardiovascular surgery. 1993 Sep;106(3):479-86.

US Food and Drug Administration. FDA Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research: Rockvile, MD, 2001

# Tables

**Table 1.** Intra and inter-assay precision and accuracy results. C.V.

| | $r^2$ | LLOQ | Recovery (%) | | Intra-assay | | | Inter-assay | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 31.25 | | | 60 | 160 | 1600 | 60 | 160 | 1600 |
| | | ng/ml | milrinone | olprinone | ng/ml | ng/ml | ng/ml | ng/ml | ng/ml | ng/ml |
| **n** | 13 | 13 | 16 | 4 | 4 | 4 | 4 | 20 | 20 | 20 |
| **Mean ± S.D. (ng/ml)** | 0.9975 ± 0.0028 | 31.2 ± 0.7 | 85.2 ± 3.1 | 63.5 ± 1.8 | 58.2 ± 0.7 | 151.0 ± 6.8 | 1587.6 ± 35.8 | 57.0 ± 1.3 | 170.1 ± 12.9 | 1599.5 ± 115.8 |
| **C.V. (%)** | | 2.3 | | | 1.2 | 4.5 | 2.3 | 2.3 | 7.6 | 7.2 |
| **% bias** | | -0.1 | | | -2.9 | -5.6 | -0.8 | -5.0 | 6.3 | 0.0 |

# Figures

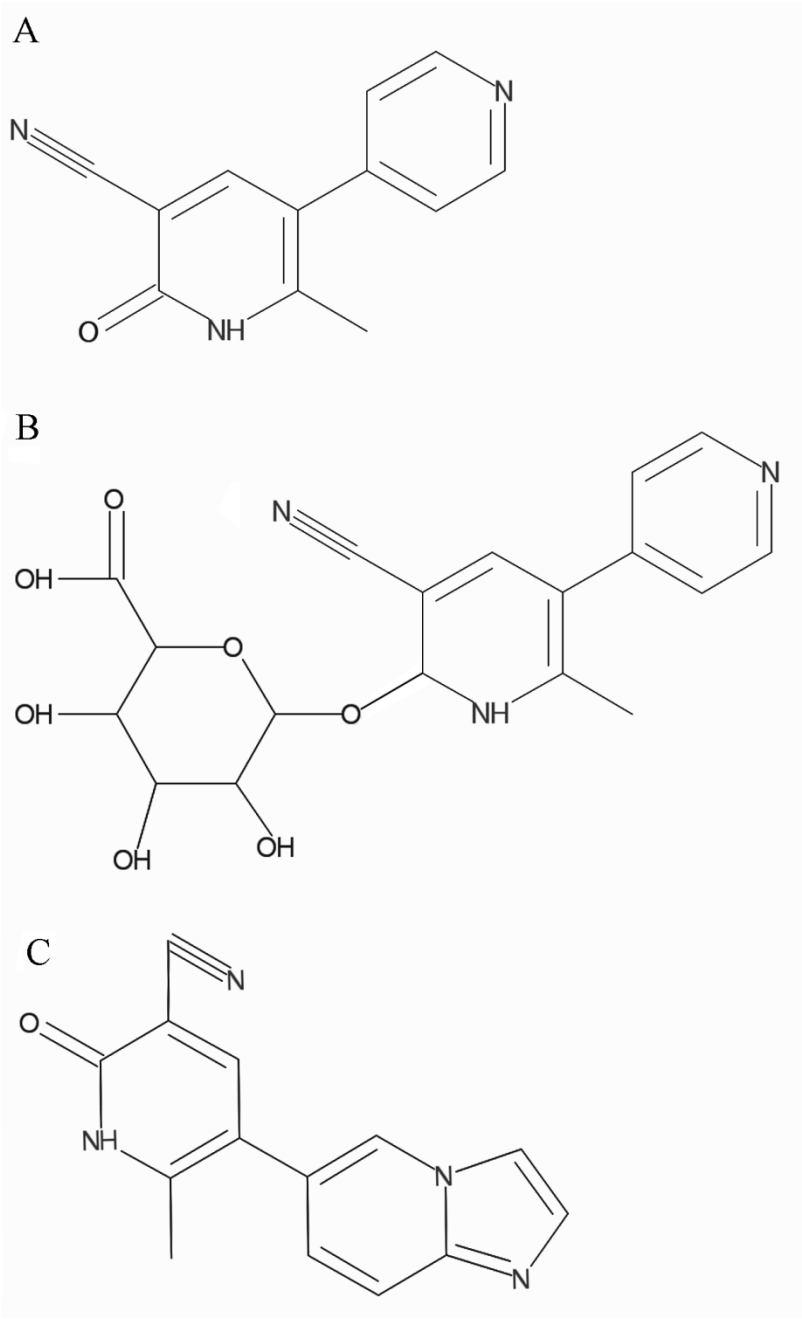


**Figure 1.** Chemical structures of A, milrinone, B, the O-glucuronic acid milrinone conjugate and C, the internal standard olprinone.

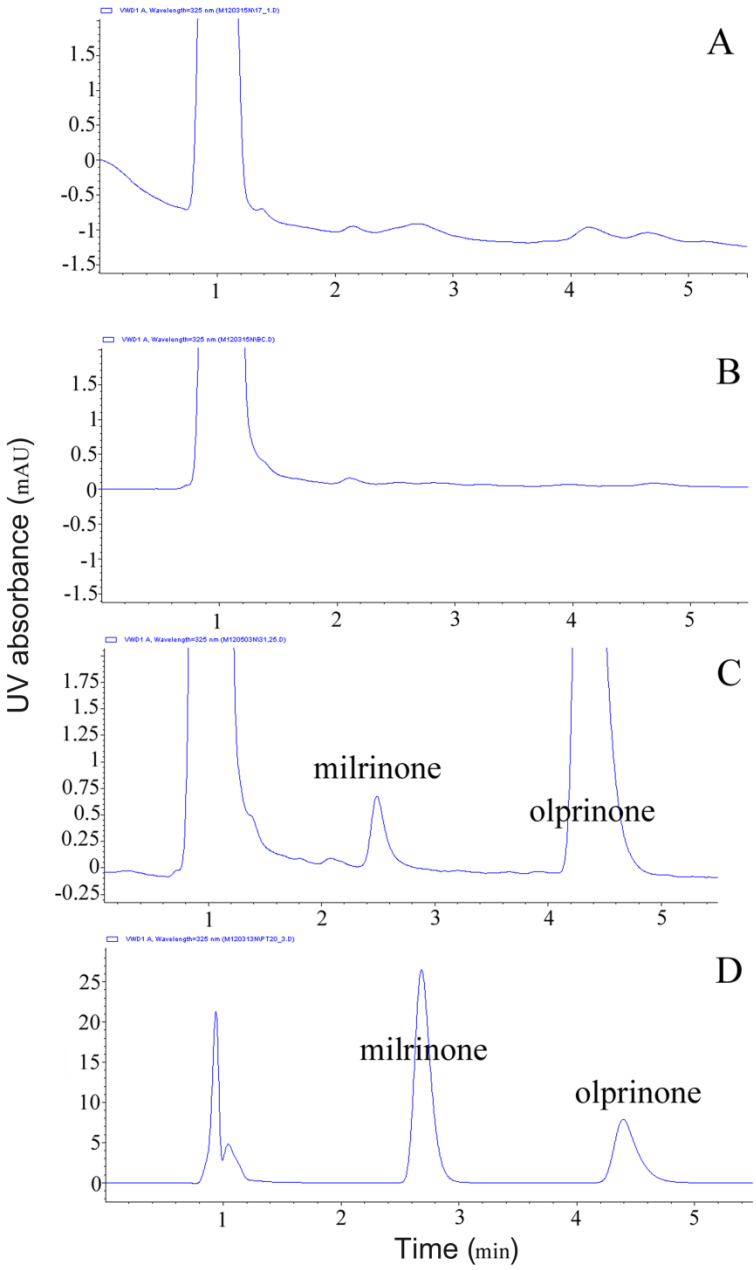


**Figure 2.** Typical HPLC chromatograms of urinary extracts from (a) blank subject sample, (b) blank cardiac patient sample, (c) milrinone-spiked (31.21 ng/ml, LLOQ) urine calibration standard (d) cardiac patient sample after inhalation of a 5 mg milrinone dose over 20 minutes (1278.5 ng/ml).

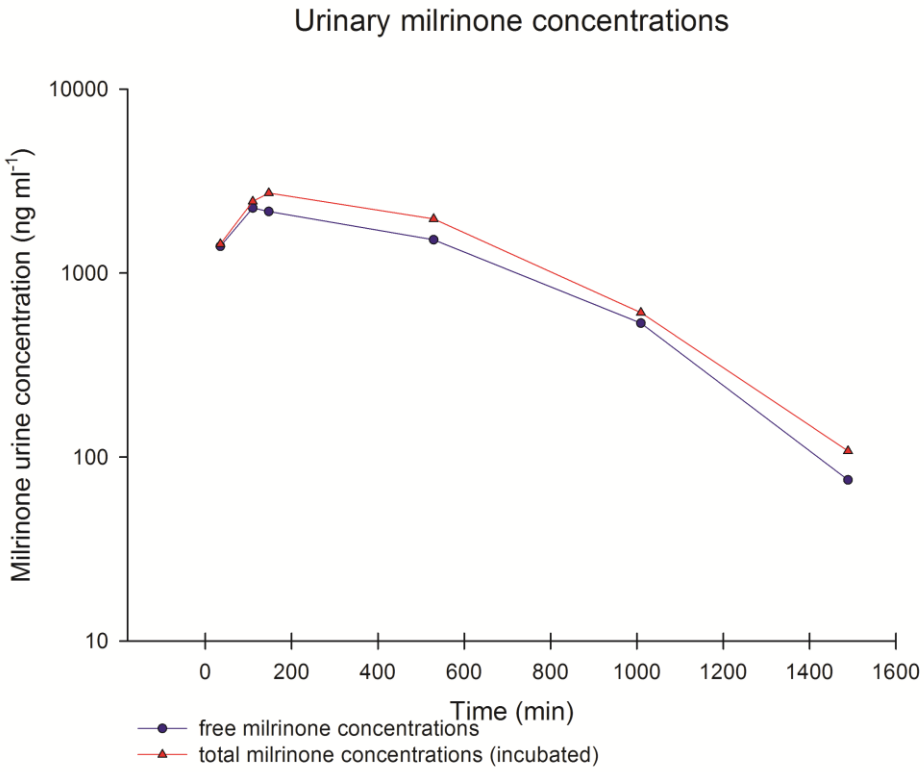


**Figure 3.** Urinary pharmacokinetic profile of a patient having received 5 mg of milrinone inhaled over 20 min.