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The effects of administration of a nuclear factor κB inhibitor on pulmonary
endothelial dysfunction after cardiopulmonary bypass:
impact on oxygenation and hemodynamics and development of therapeutic and
preventive modalities in a porcine model

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Les effets sur la dysfonction endothéliale pulmonaire de l'administration d'un inhibiteur du facteur nucléaire κB: impact sur l'hémodynamie et l'oxygénation et le développement de modalités thérapeutiques et préventives dans un modèle porcin.

Rosu C, Denault AY, Carrier M, Perrault LP

Introduction: La circulation extracorporelle (CEC) peut entraîner une dysfonction endothéliale pulmonaire et l'hypertension pulmonaire. Le SN50 agit au niveau de la signalisation cellulaire pour prévenir ces réactions à la CEC et pourrait renverser la dysfonction endothéliale pulmonaire post-CEC sans effets néfastes sur l'hémodynamie.

Méthodes: Quatre groups de porcs ont reçu un parmi quatre traîtements avant de subir 90 minutes de CEC et 60 minutes de reperfusion: (1) milrinone nébulisé; (2) sildenafil nébulisé; (3) placebo nébulisé; et (4) SN-50 intraveineux. Un monitoring hémodynamique invasif a été utilisé. La réactivité vasculaire des artères pulmonaires de deuxième ordre a été évaluée face à l'acétylcholine et la bradykinine.

Résultats: Le sildénafil produit une augmentation significative de la pression de l'artère pulmonaire (PAP) moyenne à 60 minutes de reperfusion par rapport au début de la chirurgie. Les relaxations dépendantes de l'endothélium face à la bradykinine étaient meilleurs dans les groupes milrinone et SN-50 et surtout dans le groupe sildénafil par rapport au groupe placébo. Le SN-50 produisait de moins bonnes relaxations dépendantes de l'endothélium face à l'acétylcholine que les autres traitements incluant placébo.

Conclusion: Le sildénafil prévient mieux la dysfonction endothéliale pulmonaire que les autres traitements. Les bénéfices du SN-50 sont possiblement sous-estimés vu que la dose n'a pas pu être ajustée à la durée de CEC. Le sildenafil inhalé mérite une étude plus importante chez l'humain et le SN-50 dans un model de CEC animal.

Dysfonction endothéliale – hypertension pulmonaire – circulation extracorporelle– facteur nucléaire κB – inhibiteurs de la phosphodiestérase

The effects of administration of a nuclear factor κB inhibitor on pulmonary endothelial dysfunction after cardiopulmonary bypass: impact on oxygenation and hemodynamics and development of therapeutic and preventive modalities in a porcine model.

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Background: Cardiopulmonary bypass (CPB) can lead to pulmonary endothelial dysfunction and consequent pulmonary hypertension. The novel agent SN-50 acts at the level of the transduction pathway to prevent these responses and may limit or reverse post-CPB pulmonary endothelial dysfunction and pulmonary hypertension without the untoward effects on hemodynamics seen with other known therapies.
Methods: Four groups of Landrace-Yorkshire swine that received one of four treatments before undergoing 90 minutes of normothermic CPB and 60 minutes of reperfusion were compared: (1) Nebulized milrinone; (2) nebulized sildenafil; (3) placebo consisting of nebulized NaCl solution; and (4) intravenous SN-50. Invasive hemodynamic monitoring was used throughout all experiments. Vascular reactivity of second-degree pulmonary arteries was evaluated in response to acetylcholine and bradykinin.
Results: Sildenafil produced a significant increase in mean pulmonary artery pressure (PAP) at 60 minutes after CPB compared to baseline. Both the sildenafil and milrinone groups had increased mean PAP/MAP ratio at 60 minutes after CPB compared to baseline, however this ratio was not different between the groups. Endothelial-dependent relaxations to bradykinin were improved in the SN-50 and milrinone groups and especially the sildenafil group as compared to placebo. SN-50 produced worse endothelium-dependent relaxations in response to acetylcholine compared to the other groups including placebo.
Conclusion: Sildenafil better prevented pulmonary endothelial dysfunction than all other treatments. The improvements seen with SN-50 may be suboptimal as dose could not be titrated to length of CPB. Inhaled sildenafil and SN-50 both merit further study in human trials and animal models, respectively.

Endothelial dysfunction – pulmonary hypertension – cardiopulmonary bypass – nuclear factor κB – phosphodiesterase inhibitors

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ABBREVIATIONS

5-HPETE: 5-hydroperoxyeicosatetraenoic acid

12-HHT: 12-hydroxyheptadecatrienoic acid

15-HETE: 15-hydroxyeicosatetraenoic acid

20-HETE: 20-hydroxy-5,8,11,14-eicosatetraenoic acid

A_I: Angiotensin-I

A_{II}: Angiotensin-II

ACE: Angiotensin-converting enzyme

AMP: Adenosine monophosphate

ACh: Acetylcholine

ACT: Activated coagulation time

ADH: Antidiuretic hormone

ADP: Adenosine diphosphate

ARDS: Acute respiratory distress syndrome

AT: Angiotensin

AT_1: Angiotensin receptor type 1

AT_2: Angiotensin receptor type 2

ATP: Adenosine triphosphate

AVP: Arginine vasopressin

B_1: Bradykinin receptor type 1

B_2: Bradykinin receptor type 2

BAL: Bronchoalveolar lavage

BET-1: Big endothelin-1

BH_4: Tetrahydrobiopterin

BK: Bradykinin

BK_{Ca}: Calcium-sensitive potassium channel

cAMP: Cyclic adenosine monophosphate

CABG: Coronary artery bypass grafting

cGMP: Cyclic guanosine monophosphate

COX: Cyclooxygenase

CPB: Cardiopulmonary bypass

DAG: Diacyl-glycerol

DNA: Deoxyribonucleic acid

DHCA: Deep hypothermic circulatory arrest

ECE: Endothelin-converting enzyme

EDHF: Endothelium-derived hyperpolarizing factor

EDRF: Endothelium-derived relaxing factor

EET: Epoxyeicosatrienoic acid

EFA: Essential fatty acid

EGF: Endothelial growth factor

ELAM: Endothelial leukocytes adhesion molecule

eNOS: Endothelial nitric oxide synthase

ERA: Endothelin-1 receptor antagonist

ET-1: Endothelin-1

ET_A: Endothelin receptor type A

ET_B: Endothelin receptor type B

F1.2: Prothrombin fragment

FRC: Functional residual capacity

GPCR: G-protein couple receptor

H_2O_2: Hydrogen peroxide

HMWK: High-molecular-weight kininogen

ICAM-1: Intercellular adhesion molecule-1

ICU: Intensive care unit

IGF-1: Insulin-like growth factor-1

IκB: inhibitor of κB

IKK: IκB kinase

IL: Interleukin

inhNO: Inhaled nitric oxide

iNOS: Inducible nitric oxide synthase

IP_3: Inositol 1,4,5-trisphosphate

IR: Ischemia-reperfusion

JGA: Juxtaglomerular apparatus

L-NMMA: N^G-monomethyl-L-arginine

LT: Leukotriene

LV: Left ventricle

MAP: Mean arterial pressure

N_2O_3: Dinitrogen trioxide

NADPH: Nicotinamide adenine dinucleotide phosphate

NE: Norepinephrine

NF-κB: Nuclear factor kappaB

nNOS: Neuronal nitric oxide synthase

NO: Nitric oxide

NO_2: Nitrogen dioxide

NOS: Nitric oxide synthase

O_2^-: Superoxide

OH^-: Hydroxyl radical

$ONOO^-$: Peroxynitrite

PAF: Platelet activating factor

PAH: Pulmonary arterial hypertension

PAI-1: Plasminogen activator inhibitor-1

PAP: Pulmonary artery pressure

PDE: Phosphodiesterase

PDGF: Platelet-derived growth factor

PDTC: Pyrrolidine dithiocarbamate

PE: Phenylephrine

PEEP: Positive end-expiratory pressure

PGE_2: Prostaglandin E_2

PGF_1: Prostaglandin H_2

PGH_2: Prostaglandin H_2

PGI_2: Prostaglandin I_2, Prostacyclin

PHTC: Pulmonary hypertensive crisis

PIP_2: Phosphatidyl 4,5-bisphosphate

PMN: Polymorphonuclear cell

ppm: Parts per million

PSGL-1: P-selectin glycoprotein ligand-1

PVR: Pulmonary vascular resistance

RAP: Right atrial pressure

RAS: Renin-angiotensin system

RHD: Rel homology domain

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

RV: Right ventricle

RVEF: Right ventricular ejection fraction

SNP: Sodium nitroprusside

SVR: Systemic vascular resistance

TNF: Tumor necrosis factor

t-PA: Tissue plasminogen activator

TPG: Transpulmonary gradient

TXA_2: Thromboxane A_2

TXB_2: Thromboxane B_2

V_1: Vasopressin receptor type 1

V_{1a}: Vasopressin receptor type 1a

V_2: Vasopressin receptor type 2

VCAM-1: Vascular cell adhesion molecule-1

VSMC: Vascular smooth muscle cell

WHO: World Health Organization

Acknowledgements

FIRST CHAPTER

INTRODUCTION

Since the introduction of cardiopulmonary bypass into clinical practice in 1953 with John Gibbon's successful closure of an atrial septal defect using cardiopulmonary byass (CPB) [7], millions of patients around the world have undergone successful open-heart surgery with CPB. However, while permitting the execution of complex and often life-saving surgery on the heart and great vessels, cardiopulmonary bypass induces significant disturbances in hemostasis, electrolyte balance and rheology, vascular autoregulation, and generally minor disturbances in most organ systems, as well as inducing a systemic inflammatory response. These effects may lead to grave complications, including post-CPB pulmonary hypertension with resultant right ventricular failure, cardiogenic shock, multiorgan dysfunction, and death. This infrequent yet often catastrophic complication is poorly understood and remains a therapeutic challenge.

Vascular endothelium has long been known to play roles in metabolism, coagulation, and transport. Furchgott and Zawadzki's [8]discovery in 1980 of the obligatory role of the endothelium in the acetylcholine-induced relaxation of isolated arteries presented an additional function for these cells as modulators of the contractions and relaxation of the surrounding vascular smooth muscle. Endothelial cells regulate basal vascular tone as well as smooth muscle reactivity in response to mechanical stimuli and a variety of neurohormonal mediators via the production and release of relaxant and contracting factors. In time, physiologists and pharmacologists discovered not only the significance of endothelial function in normal cardiovascular physiology but also notably the importance of disturbed endothelial function in many cardiovascular disorders, including atherosclerosis,

hypertension, heart failure, cardiac allograft rejection, pulmonary hypertension, and others [9]. Increasing understanding of the role of endothelial dysfunction in post-CPB pulmonary hypertension has slowly lead to more tailored approaches and therapies. A number of pharmacological agents have been developed to combat pulmonary hypertension in this and other contexts-including intravenous, and later inhaled, phosphodiesterase inhibitors, inhaled nitric oxide, endothelin inhibitors, and prostaglandins. Their efficacy has often been found to be limited while some have repercussions on the systemic circulation that prevent optimal usage. Alternative therapies have been sought and glucocorticoids have shown promise in the prevention of post-CPB pulmonary hypertension in experimental animals [10]. Glucocorticoid effects are however non-selective and may have unintended negative effects and consequently efforts are being made to develop agents that act more selectively downstream in the signal transduction pathway targeted by glucocorticoids. Several agents, all targeting nuclear-factor κB, have shown efficacy in different animal models [11-13].

SECOND CHAPTER

THE VASCULAR ENDOTHELIUM

Endothelial Cell Anatomy

The internal structure of an endothelial cell is no different from the majority of other human cells. A nucleus and a number of other organelles are found within the cytoplasm and are all contained within a phospholipid bilayer membrane. This cell membrane is crossed by complex proteins that may serve as receptors for blood-borne ligands or as transmembrane channels that allow the passage of substances into and out of the cell. It lies as a single monocellular layer separating the circulating blood from the deeper layers of the vascular wall. Also, contractile proteins that give the cell motor activity traverse the cytoplasm. These include actin, myosin, tropomyosim, and α-actin and they permit conformational changes of the cell in response to specific stimuli such as shear stress, which causes endothelial cells to flatten and align in the direction of blood flow [14].

Normal Endothelial Cell Functions

Vascular endothelial cells are very active metabolically, producing a variety of vasoactive mediators creating a delicate balance between vasodilatation and vasoconstriction and between thrombosis and anticoagulation. Production and release of nitric oxide (NO) is central to the regulatory role of endothelium, as the final common pathway in a number of important homeostatic mechanisms [9]. Through the production and secretion of NO and other molecules, endothelium inhibits the contraction, migration, and proliferation of vascular smooth muscle cells (VSMC) [9, 15].

The endothelium also plays an important role in the prevention of inappropriate thrombosis, through the following mechanisms: the surface expression of ecto-

adenosine phosphatase, which degrades adenosine diphosphate thus inhibiting platelet aggregation, heparan sulfate proteoglycan, a cofactor for antithrombin III, and thrombomodulin as well as the secretion of soluble factors such as tissue-factor pathway inhibitor, tissue-plasminogen activator, and the platelet-inhibitors NO and prostacyclin [16]. Simultaneously, the endothelium produces coagulation factors such as von Willebrand factor and tissue factor and the antifibrinolytic plasminogen activator inhibitor-1 (PAI-1) in order to maintain an hemostatic equilibrium.

Inflammation is also under the regulatory influence of the endothelium. Leukocyte migration and adhesion to the endothelium is downregulated by NO [9]. The dimension of the intercellular space is determined by contractions and relaxations of the junction-associated filament [17] system. Pro-inflammatory cytokines, reactive oxygen species, thrombin, platelet activating factor (PAF), increased calcium concentration in ischemic conditions, adenosine triphosphate (ATP) exhaustion, and other toxic substances all disturb the functioning of the FAU system and increase endothelial permeability [14]. Cyclic adenosine monophosphate (cAMP) and cyclic guanine monophosphate (cGMP) are intracellular second messengers that prevent intercellular separation, acting in the same manner as nitrates and their derivative NO [14].

Control of Vascular Tone

Endothelial cells also play a major role in the modulation of vascular tone. Many of the substances they synthesize and produce have vasorelaxant or vasoconstrictive properties. These substances, majoritarily vasorelaxant, include NO (the once-

unidentified endothelial-derived relaxing factor [EDRF]), prostacyclin (also called prostaglandin-I_2), the still unidentified endothelium-derived hyperpolarizing factor (EDHF), and others. The endothelium can also modulate VSMC activity in response to substances that it is itself reactive to. For example, blood-borne serotonin is almost entirely contained within circulating platelets and it is released during platelet aggregation. While most blood vessels will contract in response to serotonin, the endothelium is simultaneously triggered to produce an inhibitory signal to counteract this contraction [18]. Endothelial cells may detect these stimuli through cell membrane receptors, which lead to activation or inhibition of intracellular signaling pathways. Acetylcholine (ACh) and bradykinin (BK) are other important autacoids that stimulate endothelium-dependant relaxation. A number of endothelial-derived agents contribute to resting vascular constrictor tone including renin, angiotensin-1, and endothelin while neurohormonal agents such as norepinephrine, epinephrine, and vasopressin, while not synthesized by the endothelium, exert similar effects [19]. Vasocontrictor free radical species such as superoxide anion are also generated.
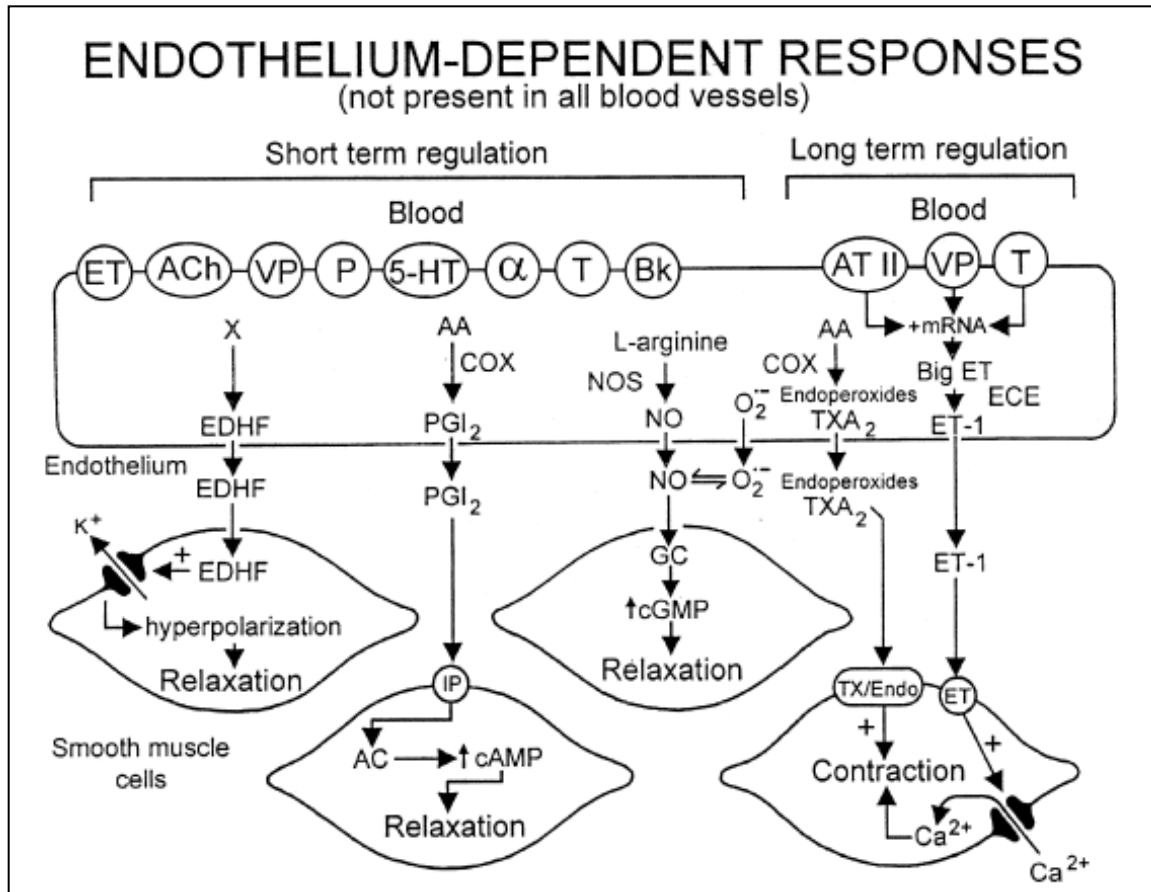
Figure 1: Variety of endothelium-derived relaxing and contracting factors.

(Reproduced with permission from the author) [1]

Control of Growth of Vascular Smooth Muscle Cells

Another important function of the endothelium is its regulation of the proliferation

of vascular smooth muscle. Its control over this process is through secretion of some

of the same substances that are necessary for control of vascular tone: NO and

prostacyclin. Nitric-oxide donors have been shown *in vitro* to inhibit smooth muscle

cell proliferation, through the cGMP-induced activation of cAMP-dependent protein

kinase [20]. This effect has been shown to be potentiated by sildenafil through its

inhibitory action on phosphodiesterase-5 [21]. Prostacyclin has also been long

known to inhibit vascular SMC proliferation and studies have shown the

antiproliferative effects of prostaglandins to be related to increased intracellular cAMP [22, 23]. Also, certain species of heparan sulfate, a proteoglycan, possess antiproliferative properties and are produced by endothelial cells as well as VSMC [24]. Pro-proliferative compounds secreted by endothelial cells include endothelin-1, angiotensin-II, platelet-derived growth factor (PDGF), basic fibroblast growth factor, and insulin-like growth factor-1 (IGF-1) [25, 26].

Vasorelaxant Factors
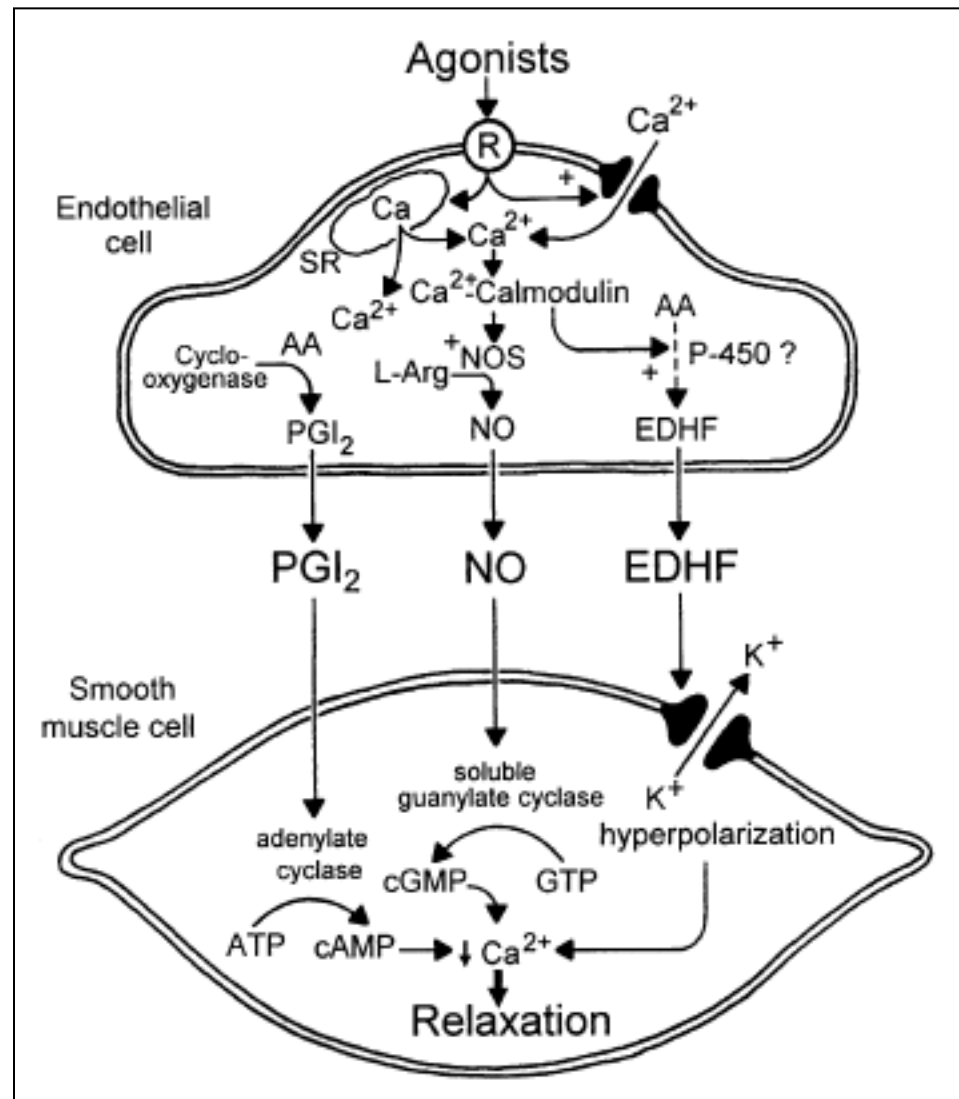
Figure 2. Release of endothelium-derived relaxing factors and their effects on

vascular smooth muscle cells. (Reproduced with permission from the author) [2]

Nitric Oxide (NO)

Once thought to be simply a pollutant gas, the identification of nitric oxide as the

agent responsible for the activity of EDRF was a major discovery in the field of

cardiovascular medicine [27-29]. Produced by the endothelial cells, NO is quite

labile in solution and has a short half-life (as short as 4 seconds) [30]. However, due to its low molecular weight and lipophilic properties, it is able to diffuse easily across cell membranes [14]. In addition to its role in endothelial function and cardiovascular homeostasis, NO also acts as a neurotransmitter and plays a role in the immune response as a cytostatic/cytotoxic agent. It is synthesized in the endothelial cells from L-arginine and molecular oxygen by nitric oxide synthase (NOS), yielding citrulline as a by-product. A number of cofactors are required for nitric oxide synthesis, including nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide, flavin adenine dinucleotide, tetrahydrobiopterin (BH4), and calmodulin [9]. After diffusing from the endothelium into the underlying VSMC, it activates guanylate cyclase, causing an increase in the cGMP concentration [31]. It is this second messenger that mediates many of the effects of NO, inducing vascular smooth muscle relaxation through cyclic-GMP dependent phosphorylation of the myosin light chain and decreases in cytosolic calcium concentration [32, 33]. A number of other intracellular molecules may interact with NO, such as heme and other iron-containing proteins, DNA, and thiols. Nitric oxide may also affect the enzymes of the respiratory chain, thus altering mitochondrial ATP generation. Superoxide and other oxygen free radicals are powerful inactivators of NO, leading to the generation of peroxynitrite which is rapidly converted to nitrate [9, 34]. Hemoglobin and myoglobin are also potent inactivators of NO, with methemoglobin and metmyoglobin relatively weaker inactivators, and all produce reversible inactivation [35].

Three isoenzyme forms of NOS have been identified: neuronal-NOS (nNOS or NOS-I), inducible-NOS (iNOS or NOS-II), and endothelial-NOS (eNOS or NOS-III) [9, 14, 30]. The genes that encode for these enzymes are on chromosomes 12, 17, and 7, respectively [36]. The two constitutive forms of NOS, eNOS and nNOS, are stimulated by increases in intracellular calcium and calmodulin and produce low levels of NO. The third isoform, inducible NOS (iNOS), is found usually within macrophages but may be found within endothelial cells as well and steadily produces important quantities of NO when stimulated. This enzyme is calcium-independent and its expression is seen mainly in states of inflammation and infection where it is stimulated by cytokines, such as tumor necrosis factor, or bacterial endotoxin. Expression and production of this form of the inducible enzyme is inhibited by glucocorticoids, unlike the constitutive forms, while the enzymes' activity is unresponsive to this treatment [37, 38]. However, it is eNOS, not iNOS, which is responsible for the continuous basal synthesis of nitric oxide by endothelial cells that maintains normal vascular tone. N^G-monomethyl-L-arginine (L-NMMA), an analogue of L-arginine that acts as a reversible inhibitor of NOS, has been used in experimental models to demonstrate the importance of basal NO secretion from the endothelium. Intravenous infusion of L-NMMA induces a dose-dependent increase in blood pressure in experimental animals and infusion of L-NMMA into human brachial artery cause significant dose-dependent vasoconstriction [9, 39]. Conversely, in the venous system of a variety of animals and of humans, NOS inhibition has little effect on vascular tone, signifying a small role for basal NO production in the maintenance of resting venous tone [40]. eNOS is preferentially

found within caveolae in endothelial cells after it has undergone post-translational acylation and it is negatively regulated by caveolin [41]. Stimulation of endothelial cells by agonists that mobilize intracellular calcium leads to dissociation of the NOS-caveolin complex and binding of calcium-calmodulin to NOS, thus activating NO synthesis [42].

A number of factors induce endothelium-dependent vasodilatation through the production of NO. One of the most important stimuli is shear stress, which is related to blood velocity [43]. Activation of NOS by shear stress occurs through a calcium-independent, protein tyrosine kinase-dependent mechanism as well as through more rapid changes in calcium, potassium, and chloride transmembrane currents [9, 44]. A number of chemical substances induce endothelium-dependent relaxations through release of NO, such as acetylcholine, bradykinin, substance P, and the calcium ionophore A23187 [9, 29, 45]. The principal pathway for the action of these substrates (other than the calcium ionophore) is through G-protein dependent activation of a phospholipase C leading to increases in intracellular free calcium due to mechanisms resulting from hydrolysis of phosphatidylinositol-4,5-biphosphate [46].

Prostacyclin (PGI$_2$)

Eicosanoids are signaling molecules made by the oxidation of twenty-carbon essential fatty acids (EFAs). They can be classified into four families: prostaglandins, prostacyclins, leukotrienes, and thromboxanes. Arachidonic acid, an omega-6 fatty acid, is the most abundant and most important of the three eicosanoid precursor

EFAs. After arachidonic acid has been mobilized from the cellular membrane by phospholipase-A_2, the enzyme cyclooxygenase oxidizes it into prostaglandin-H_2 (PGH_2) in a two-step process. PGH_2 is subsequently transformed into prostacyclin (also known as prostaglandin-I_2) by the endoplasmic reticulum membrane protein prostacyclin synthase. While prostacyclin synthase activity is not regulated by intracellular calcium concentration, phospholipase-A_2 activity, and therefore the generation of prostacyclin precursors, is calcium-dependent [46]. Prostacyclin has potent antithrombotic effects through inhibition of platelet activation and vasodilatory effects. It is actively generated within endothelial cells in response to sodium arachidonate, thrombin, the ionophore A23187, and trypsin and accounts for the vast majority of the prostanoids produced [47, 48]. Their action, both on platelets and on VSMC, depends on the presence of receptors for this molecule [49]. Prostacyclin-receptors within the VSMC are coupled to adenylate cyclase, leading to increased cAMP levels [50]. Elevated cAMP increases calcium extrusion from the cytosol, inhibiting the contractile machinery [51, 52]. Prostacyclin-dependent stimulation of ATP-sensitive potassium channels produces hyperpolarization of the cell membrane and contributes in small part to the relaxation induced by prostacyclin [53]. Prostacyclin also contributes to endothelium-dependent relaxation through stimulation of NO release by endothelial cells as well as acting synergistically with NO on VSMC [54]. The mechanism underlying this synergy is cGMP-induced inhibition of phosphodiesterase 3, leading to increased levels of cAMP [55].

Endothelium-Derived Hyperpolarizing Factor

Endothelium-dependent relaxations that are resistant to antagonists of the L-arginine–nitric-oxide pathway and cyclooxygenase inhibitors exist and appear to act through membrane hyperpolarization. Acetylcholine was the first compound demonstrated to induce release of the endothelium-derived, albeit unidentified, hyperpolarizing factor (EDHF) responsible for this effect [56]. Bradykinin and shear stress have also been shown to induce EDHF production in endothelial cells. EDHF release in response to acetylcholine is mediated by M_1-muscarinic receptors, whereas NO release is mediated by M_2-muscarinic receptors [57]. This hyperpolarization appears to be mediated by the opening of K^+ channels, given that its effect is mimicked by the K^+ channel opener cromakalim and inhibited by increasing extracellular potassium concentration [58]. Inhibition of acetylcholine-induced relaxation by tetraethylammonium and not glibenclamide suggests that EDHF acts on calcium-sensitive potassium channels (BK_{Ca}) rather than ATP-sensitive potassium channels [59]. Relaxation by EDHF is more prominent in arteries with smaller diameters, unlike NO, which is greater in larger vessels [60]. Despite having identified these characteristics of EDHF, its identity remains uncertain, in part due to heterogeneity between species and between different tissues. However several substances have been proposed as EDHF. Epoxyeicosatrienoic acids [61] are cytochrome P450 metabolites of arachidonic acid and, particularly the 5,6-EET metabolite, have been shown to have characteristics and activities very similar to EDHF [62]. The endogenous cannabinoid, or 'endocannabinoid', anandamide is another arachidonic acid metabolite formed via the action of a transacylase enzyme that has been suggested as an EDHF [63]. Other

reports suggest however that the physiological properties of anandamide are not identical to those of EDHF [64]. Edwards *et al.* demonstrated that an increase in potassium in the extracellular space between endothelial and VSMC can mimic the effects of EDHF by stimulating an inwardly rectifying potassium current in vascular myocytes (1998).

Adenosine

Adenosine is generated in metabolically active tissues as the result of hydrolysis of adenosine monophosphate (AMP) in order to generate energy when the more phosphorylated adenosine moieties (adenosine diphosphate [ADP] and ATP) are insufficient to produce the energy required in cellular metabolism. It may also be released from aggregating platelets. Adenosine itself directly causes relaxation of vascular smooth muscle through surface receptor-mediated induction of guanylate cyclase [65]. It can also induce endothelium-dependent relaxations by stimulating endothelial cell production and release of NO [66]. While adenosine and AMP may cause endothelium-independent relaxation, ATP and ADP both require the presence of the endothelium in order to provoke relaxations [67]. Adenosine may also be directly generated by the endothelium, leading to autocrine stimulation [68].

Bradykinin

Bradykinin is a peptide of the kinin group of proteins that are derived from precursor kininogens. Along with acetylcholine, it has been long known to cause endothelium-dependent vasodilation [69]. Bradykinin may be generated by proteolytic cleavage of its precursor high-molecular-weight kininogen (HMWK) by

the enzyme kallikrein or from the proteolysis of kallidin by the enzyme aminopeptidase B [70]. Kallikrein is found both in plasma, where it also plays an important role in hemostasis, and within tissues [71]. Bradykinin degradation is accomplished by three different enzymes: angiotensin-converting enzyme (ACE), aminopeptidase P, and carboxypeptidase N. Notably, part of the antihypertensive effect of the ACE inhibitor class of medications is by preventing the loss of the vasodilatory stimulus of bradykinin. Some of the vasorelaxant effect of bradykinin appears to be through generation of the relaxing factor prostacyclin [67]. Synthesis of this molecule by endothelial cells is the result of cytosolic phospholipase A_2 activation through an increase in intracellular calcium concentration and through protein kinase C-dependent mechanisms [72]. However, much of the endothelium-dependent relaxant activity of bradykinin is through the generation of NO [27]. Bradykinin exerts its activity through the B_1 and B_2 bradykinin receptors, both of which are G-protein coupled receptors (GPCR). Stimulation of endothelial B2-receptors leads to activation of multiple transduction pathways which increase intracellular calcium, with some pathways doing so directly while other pathways produce the same effect in a more roundabout way [71]. In particular, activation of phospholipase C leads to cleavage of inositol 1,4,5-trisphosphate (IP_3) from phosphatidyl 4,5-bisphosphate (PIP_2), which stimulates calcium channels on the endoplasmic reticulum to release calcium. Ultimately, the increase in endothelial intracellular calcium is the stimulant for NOS activity.

Histamine

Histamine is a molecule that participates in the local immune and inflammatory response to cellular infection or injury. It is produced and release at the tissue level by mast cells and by basophils in the blood. Its principal actions on endothelium are through the H_1 histamine receptors, which lead to vasodilation and increased cellular permeability, the purpose of which is to allow leukocyte extravasation.

Vasoconstricting Factors

While the discussion has until now almost exclusively concerned vasorelaxant factors, endothelium also produces vasoconstrictor substances, which are necessary in its principal task of maintaining vascular homeostasis. These vasoconstricting factors include endothelin-1, the components of the Renin-Angiotensin system (the angiotensins and renin), the arachidonic acid metabolites prostaglandin H_2 and thromboxane A_2, and reactive oxygen species such as peroxynitrite, superoxide, and hydroxyl radical. Of all of these, the physiologically most important is endothelin-1, which will be explained below.

Prostaglandins/Thromboxanes

The prostanoids are generated as the result of transformation of arachidonic acid by cyclooxygenase [73] into prostaglandin H_2, the precursor to all other prostanoids. COX exists in two isoforms, COX-1 and COX-2 also called PGH_2 synthase-1 and -2, respectively. COX appears to be present in the membranes of all cells [74]. Thromboxane A_2 (TXA_2) is produced from PGH_2 by the enzyme thromboxane A synthase 1, found in platelets, and is relatively labile, with a half-life of 30 seconds [49]. Both TXA_2 and PGH_2 share the same pharmacological properties of increasing

platelet activation and aggregation as well as constriction of VSMC and bronchial smooth muscle and are thought to share the same GPCR [49]. Notably, TXA2 and prostacyclin can be regarded as antagonist molecules, with many effects that oppose eachother. However, given that prostacyclin is the major product of COX in endothelial cells and the additional vasodilatory influence of NO and EDHF, vasoconstrictor prostanoids have little influence on vascular tone under physiological conditions [74].

Endothelin

Endothelin-1 (ET-1) was first described as a then-unknown protein vasoconstrictor found within the culture media of bovine aortic endothelial cells [75]. It was soon isolated and identified as an endothelium-derived 21-residue polypeptide that was also shown to be one of the most potent vasoconstrictors known [76]. ET-1 is the only isoform produced by endothelium and may be released in response to a number of stimuli, including hypoxia, low sheer stress, thrombin, interleukin-1, angiotensin II, transforming growth factor-β, vasopressin, and catecholamines. Other tissues that may produce endothelins are the brain, the lungs, the kidneys, and certain circulating cells such as mononuclear cells [77]. The principal producer of endothelin, however, is the vascular endothelium. The human ET-1 gene is encoded on chromosome 6 and the mature protein corresponds to the second exon [78]. The result of transcription and translation of the gene is preproendothelin-1, a 203 amino acid long protein, which is then processed into the 39-amino-acid big endothelin-1 (BET-1). BET-1 is secreted into and circulates in plasma. However, the vasoconstrictive potency of BET-1 is 1/100th that of the mature protein and thus

generally does not contribute importantly to the control of vascular tone. BET-1 is converted into the active form ET-1 by the metalloprotease endothelin converting enzyme (ECE), of which there are two forms, both sensitive to the neutral metalloprotease inhibitor phosphoramidon [77]. ECE-1 is the most important form and is present on the cell surface membrane. The plasma half-life of ET-1 is 4-7 minutes and there is significant clearance by the lungs, with 80% on the first pass [77].

Two other endothelin isoforms exist: endothelin-2 and endothelin-3. ET-1 exerts its actions through the GPCRs ET_A and ET_B. ET_A receptors are mainly found on VSMC and serve to induce a vasoconstrictive response. Binding of ET-1 to this receptor activates phospholipases A_2 and C as well as causing an increase in intracellular calcium concentration, both leading to smooth muscle cell contraction. ET_B receptors are also found on VSMC but are much more frequently found on endothelial cells and lead to the production of the vasorelaxant molecules NO and prostacyclin [79]. This effect may serve as a negative-feedback mechanism that maintains vascular tone. In fact, at physiologically low ET-1 concentrations, the secondary vasodilation induced may supercede any vasoconstrictive effect. In addition to its effects on vascular tone, endothelins appear to have mitogenic properties on vascular and airway smooth muscle [77]. While principally acting through autocrine and paracrine effects, increased plasma levels of endothelin may be found in disease states such as heart failure, pulmonary hypertension, and coronary artery disease and was demonstrated to be increased after CPB in a porcine model in a recent paper from our laboratory [80, 81].

The Influence of the Renin-Angiotensin System

The Renin-Angiotensin System (RAS) is a cascade of enzymes that culminates in the production of angiotensin-II (A_{II}). Its principal roles are in the regulation of blood pressure and intravascular fluid balance. Renin is produced by the kidney and degrades the liver-derived protein angiotensinogen into angiotensin-I (A_I). This protein is then hydrolyzed by circulating or tissue ACE into A_{II}. Other enzymes including Chymase, Carboxypeptidase, Cathepsin G and Tonin can all generate A_{II} from A_I independently of ACE, and angiotensinogen can also be directly hydrolyzed to A_{II} by non-renin enzymes, such as tissue plasminogen activator (t-PA), Cathepsin G and Tonin [14]. Also, substrates other than A_I may be hydrolyzed by ACE due to structural similarities. These substrates include bradykinin (thus further enhancing the vasoconstrictive activity of the RAS), substance P, enkephalins, neurotensin, and takynine.

The stimulus for secretion of renin into the circulation by the kidney is a decreased flow rate of the filtrate received by the macula densa of the distal tubule or by a decreased sodium concentration of the filtrate. The macula densa stimulates the specialized VSMC of the afferent arteriole, known as the juxtaglomerular apparatus [82], to produce and secrete renin. Release of renin by the JGA may also be stimulated by autonomic nervous system-induced activation of β_1-receptors.

While traditionally considered to be solely a systemic circulatory control mechanism, more recent data has shown that there is a tissue-level RAS that complements the plasma RAS in maintenance of homeostasis. The endothelial cells generate and modify angiotensins at the local tissue level [83]. Circulating

components of the RAS may also be taken up by endothelial cells and stored for later use in the tissue RAS [84]. In addition to effects on vascular tone, the tissue RAS has been found in a number of organs and may have a role in cell growth and differentiation as well as apoptosis.

Angiotensin-II

Angiotensin-II, as previously described, is the product of hydrolysis of A_I by ACE. A_{II} is the principle ligand for the angiotensin (AT) receptors, a group of GPCR. While four different subtypes of AT receptors have been described, the most physiologically important receptors (and also the best characterized) are the AT_1 and AT_2 receptors. AT_1 receptor action is mediated through immediate activation of phospholipase C with generation of inositol triphosphate and intracellular calcium release, activation of the mitogen-activated protein kinase system within minutes, and activation of nuclear transcription factor pathways within hours [85]. Generation of diacyl-glycerol (DAG) by phospholipase C leads to activation of the protein kinase C signaling pathway. This pathway has substrates that include proteins important for cellular proliferation. Hence, among the effects of the AT_1 receptor are VSMC hypertrophy and cardiomyocyte hyperplasia. These actions are through transactivation of cellular receptors for endothelial growth factor (EGF) and platelet-derived growth factor) [86]. Other effects include vasoconstriction, aldosterone synthesis and secretion, increased vasopressin secretion, increased peripheral noradrenergic activity, decreased renal blood flow, renal renin inhibition, renal tubular sodium reuptake, modulation of central sympathetic nervous system activity, cardiac contractility, a role in central osmocontrol, and extracellular matrix

formation [87]. AT_1 receptors are principally found in cardiovascular cells (VSMC and cardiac myocytes) as well as several other organs, including the brain, adrenal cortex, kidneys, and lungs. AT_2 receptors are found principally in the fetus and demonstrate significantly decreased expression after birth. In the adult, AT_2 receptor activity seems to be limited to the coronary microcirculation [88]. While its effect are less well known, they generally antagonize those of the AT_1 receptors, specifically vasodilatation, inhibition of cellular proliferation, and apoptosis [14].

Significantly less is known about the effects of A_{II} on endothelial cells compared to those on VSMC. A_{II} increases production of reactive oxygen species (ROS), specifically superoxide, through AT_1 and AT_2 receptor-mediated activation of NADPH oxidase [89]. Through this increase in ROS generation and other indirect mechanisms, A_{II} induces endothelial cell apoptosis. AT_1 receptor activation also directly alters eNOS function by binding to membrane-localized eNOS [90]. Conversely, AT_2 receptor activation in endothelial cells and VSMC leads to increased generation of bradykinin, which binds to B_2 receptors on both endothelial cells and vascular smooth muscle cells [90]. In the endothelial cells, the B_2 receptor increases eNOS activity leading to NO generation and VSMC relaxation. NO also leads to downregulation of transcription of AT_1 receptors [91].

Humoral Control of Circulation

Epinephrine and Norepinephrine (NE)

Epinephrine and norepinephrine are catecholamines principally produced by the chromaffin cells of the adrenal medulla and also by the postganglionic fibers of the

sympathetic nervous system. They are derived from the catecholamine dopamine, which is itself derived from the amino acid tyrosine. NE is principally a vasoconstrictor, through its action on α_1-adrenergic receptors of vascular smooth muscle. Epinephrine also has vasoconstrictive effects through stimulation of the same α_1-receptors. However, through stimulation of $\alpha2$- and $\beta2$-adrenergic receptors, epinephrine can also produce vasodilation. $\beta2$-receptor mediated vasodilatation is the result of an increase in intracellular cAMP <u>in</u> vascular smooth muscle. It has also been recently shown that $\beta2$-mediated vasodilation may have a NO-mediated component [92]. Both epinephrine and NE may also produce endothelial-dependent relaxation in certain vascular beds through the stimulation of α_2-receptors on endothelial cells [18].

<u>Vasopressin</u>

Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is a neurohypophysial hormone. It is a peptide hormone that controls the reabsorption of water in the tubules of the kidneys as well as having effects on vascular tone, principally of vasoconstriction. AVP is the product of modification of a preprohormone precursor synthesized in the hypothalamus and it is stored in the posterior pituitary gland. Secretion into the bloodstream is stimulated by increased osmolality of plasma and in response to vascular underfilling as detected by left atrium, aortic arch, and carotid sinus baroreceptors. AVP executes its vasoconstrictant action through the V_{1a}-receptor, a GPCR which activates phospholipase C. Antidiuretic activity is mediated by the V_2-receptor, also a GPCR, with subsequent stimulation of adenylate cyclase [93]. While better known for its

vasoconstricting effect, AVP has been shown to have endothelium-dependent relaxant effects in canine cerebral arteries but not in canine peripheral vessels, acting through V_1-vasopressinergic receptors [18].

<u>Reactive Oxygen and Nitrogen Species</u>

A number of oxidant byproducts are produced as a consequence of normal aerobic metabolism. These include hydrogen peroxide (H_2O_2), superoxide (0_2^-), hydroxyl radical (OH^-) as well as other molecules and they are collectively called reactive oxygen species (ROS). Reactive nitrogen species (RNS) include peroxynitrite ($ONOO^-$), nitrogen dioxide ($\bullet NO_2$), and dinitrogen trioxide (N_2O_3) derived from the reaction of NO with 0_2^-. While often thought of as entirely harmful, ROS/RNS can have beneficial effects at low concentrations by playing a role in host defense (they are produced by neutrophils and macrophages in order to destroy invading microorganisms) or in cell signaling. Production of these byproducts is almost constant but a check is kept on their activity by the simultaneous maintenance of a powerful antioxidant system. These antioxidants include superoxide dismutase, glutathione peroxidase, catalase, vitamin E, β-carotene, and vitamin C [14]. Harmful oxidative and nitrosative stress occurs when there is an imbalance between the generation of ROS/RNS and the antioxidant system and can damage cellular DNA, proteins, and lipids. Four cellular enzyme systems compose the principal sources of these molecules: NADPH oxidases, xanthine oxidase, uncoupled NOS, the cytochrome P450 system, and mitochondrial sources [94, 95]. There is significant interplay betweens these systems leading to increased free radical production as a result of feed-forward mechanisms. Ischemia-reperfusion injury is also well known

to generate free radicals. Specifically, xanthine oxidase and the cytochrome P450 system have been inculpated in the generation of free radicals seen in ischemia-reperfusion [5].

Uncoupling of eNOS is an important link between ROS/RNS and endothelial dysfunction. eNOS uncoupling occurs in the absence of L-arginine and the essential cofactor BH_4; eNOS is unable to transfer an electron to L-arginine and instead uses molecular oxygen as an electron receiver, thus generating O_2^- [96]. Oxidation of BH_4 by ROS generated by NADPH oxidase leads to eNOS uncoupling and is an example of the mentioned feed-forward mechanisms [97]. eNOS is also subject to upregulation by H_2O_2, with activation and even increased expression of the enzyme. Consequently, in a state of oxidative stress, there may be increased levels of uncoupled eNOS producing even large quantities of O_2^- [94]. While not all eNOS is uncoupled at any one time, NO levels will be subnormal as a consequence of decreased generation as well as due to reaction of NO with O_2^- yielding $ONOO^-$ [95].

Nuclear factor κB (NF-κB)

The nuclear factor kappaB (NF-κB) family is a group of structurally related DNA-binding proteins that can be found in a variety of organisms, from vertebrates to insects and even viruses. They act as transcription factors, regulating the expression of a wide variety of genes involved in many important cellular processes including angiogenesis, apoptosis, cell growth and adhesion, stress response, and innate immunity. They are early markers of inflammation and cellular activation, in particular endothelial activation. The NF-κB protein family in vertebrates consists of

the proteins p50/p105, p52/p100, c-Rel, RelA (also called p65), and RelB [98]. The

evolutionarily conserved domain through which all proteins in this family are

related, called the Rel homology domain (RHD), contains amino acid sequences

allowing DNA-binding, dimerization, and nuclear localization [98, 99]. In order to

bind DNA, NF-κB proteins must form dimers, which may be a homodimer or any

combination of NF-κB proteins as a heterodimer. Once dimerized, the NF-κB

proteins can translocate to the nucleus where they usually bind to 9-10 base pair

DNA sites. These sites, usually called κB sites, are found in the regulatory regions of

many cellular and viral promoters. Prior to being stimulated, NF-κB is usually found

within the cytoplasm in an inactive state, a state in which it remains due to

interactions with protein inhibitors of κB (IκB). The IκB family of proteins bind the

RHD of NF-κB dimers through a series of amino acid repeats, called ankyrin repeats

[98]. Proteins within this family include IκBα, IκBβ, IκBε, Bcl-3, and the C-terminal

sequences of the NF-κB precursor proteins, p105 and p100. IκB's inhibition of NF-

κB activity is through two mechanisms: by preventing nuclear translocation and

inhibiting NF-kB DNA binding. Induction of NF-κB may come as the result of a

number of stimuli, including bacteria, viruses, cytokines, oxidative stress, growth

factors, and certain chemotherapy agents. These stimuli activate the cellular kinase

complex IκB kinase (IKK) [99]. IKK is composed of three subunits: two related

kinases (IKKα and IKKβ) and a sensing/scaffold protein, IKKγ. Activation of the IKK

complex is through phosphorylation of residues in the activation loop of IKKα or

IKKβ and probably of IKKγ. Activated IKK phosphorylates IκB, which marks this

protein for degradation by the ubiquitin-proteosome pathway, or partial proteolysis

in the case of the inhibitory C-terminal sequences p105 and p100 [98, 99]. The now-uninhibited NF-κB dimer can translocate to the cellular nucleus, bind to DNA, and activate expression of the target genes. Activation of NF-κB is often transient, as a negative feedback mechanism exists whereby one of the target genes of NF-κB is that encoding IκB. Consequently, newly formed IκB can once again inactivate NF-κB and relegate this protein back to the cytoplasm. Interestingly, IκB expression is also stimulated by NO [14]. In addition to IκB, there appears to be other mechanisms through which NF-κB activity is regulated, including posttranslational modification, interaction with other transcription factors, and effects of IKK proteins on κB-containing gene promoters [98].

As previously mentioned, NF-κB is an important transcription factor in a number of biological processes. Over 300 genes are regulated by its activity and contribute to the maintenance of normal physiology. However, abnormal NF-κB activity has also been linked to a number of human diseases, including arthritis, asthma, cancer, diabetes, inflammatory bowel disease, ischemia/reperfusion damage, and sepsis. Multiple reports have found elevated expression of NF-κB in pulmonary hypertension in different animal models and in humans. In a rat model, animals exposed to prolonged normobaric hypoxic conditions were found to have significantly higher NF-κB expression in lung tissues, higher mean pulmonary artery pressures, and thicker pulmonary arteriole wall thickness than controls [100] with similar findings in a rat model of monocrotaline-induced pulmonary hypertension [101]. In humans, a clear overexpression of NF-κB was demonstrated in the pulmonary vascular structures of cystic fibrosis subjects who suffered from

pulmonary hypertension and exhibited endothelial dysfunction [102]. These same subjects were also found to have higher endothelin-1 activity compared to those without endothelial dysfunction. The study did not seek however to demonstrate the underlying mechanisms linking increased NF-κB and increased endothelin-1. However, this link was shown earlier in aortic endothelial cells, with the demonstration that ET-1 transcription is controlled by the transcription factor NF-κB [103].

NF-κB overactivity also contributes to endothelial dysfunction in circumstances unrelated to pulmonary hypertension. In particular, endothelial dysfunction in overweight or obese middle-aged and older humans was found to be associated with greater expression of NF-κB. Moreover, elevated endothelial cell NF-κB was associated with an abundance of endothelial cell nitrotyrosine, a marker of oxidative stress, suggesting that oxidative stress is the mechanism connecting NF-κB activation and endothelial dysfunction [104]. Other studies have shown endothelial cell activation and dysfunction to be through NF-κB dependent mechanisms and could be prevented by pre-treatment with an exogenous NF-κB inhibitor MG-132 [105]. Increased NF-κB activation is also found in endothelial cells having suffered from ischemia-reperfusion due to CPB [106]. In addition, the cytochrome P450-derived eicosanoid 20-hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) has been shown to stimulate NF-κB while also causing uncoupling of eNOS [107].

THIRD CHAPTER

THE PHYSIOLOGIC RESPONSE TO CARDIOPULMONARY BYPASS

One of the unique features of cardiac surgery not found in other surgical specialities is the need for a special perfusion system for the majority of cardiac surgical procedures. Before the advent of cardiopulmonary bypass, a variety of techniques were used to effect intracardiac repairs, generally in cases of congenital heart disease. Induced-hypothermia combined with cardiac-inflow occlusion was successfully used by Swan and colleagues in a number of cases [108]. During the same period, Lillehei and colleagues at the University of Minnesota used a technique called controlled cross-circulation whereby the circulation of the patient, usually a small child, was connected to that of a close relative of the same blood type, thus maintaining the circulation of the child while his/her heart was stopped and repair was performed [109]. While the development of the heart-lung machine in the form of a mechanical pump oxygenator by Gibbon and subsequent work by others to improve the machine lead to the abandonment of controlled cross-circulation, the latter technique maintained one fundamental advantage over mechanical cardiopulmonary bypass: the patient's blood does not come into contact with synthetic non-endothelialized surfaces. This contact of blood with the synthetic surfaces in the cardiopulmonary bypass circuit, as well as with tissues within the thoracic wound, triggers a potent inflammatory response that is responsible for the majority of the side effects of extracorporeal circulation. In addition to the inflammatory stimulus, the synthetic surfaces and wound tissues are also extremely strong thrombotic stimuli.

Hemostatic System

Anticoagulation

The large procoagulant surface of the cardiopulmonary bypass circuit renders extracorporeal perfusion impossible without adequate anticoagulation. The naturally occurring circulating anticoagulants, antithrombin, proteins C and S, tissue factor pathway inhibitor, and plasmin, are overwhelmed by the prothrombotic stimulus of the non-endothelialized surface resulting in production of thrombin. In order to maintain the fluidity of blood, therapeutic anticoagulation is necessary, habitually in the form of intravenous heparin. Heparin is a highly sulfated glycosaminoglycan found in a variety of animal species and is produced by basophils and mast cells. Its utility as an anticoagulant is through binding and enhancing the activity of circulating protease antithrombin-III, although in the absence of antithrombin-III it has no inherent anticoagulant activity [110]. Antithrombin-III primarily binds thrombin; its inhibitory action on factors Xa and IXa is much slower and has little effect on thrombin bound to fibrin [111] or factor Xa bound to platelets within clots [112]. Heparin also activates other blood constituents to varying degrees, including platelets, factor XII, complement, neutrophils, and monocytes [113-115]. Heparin used in clinical practice is generally derived from bovine or porcine mucosal tissues and is heterogeneous in size, with a molecular weight ranging from 5,000 to 30,000 Daltons, and thus called unfractionated heparin. Despite the development of a variety of novel anticoagulants, the advantage of unfractionated heparin remains in its ease of reversibility with protamine.

Protamine is a strongly alkaline arginine-rich polycationic protein extracted from salmon sperm, and also found in the sperm of a variety of animals and humans. It is found principally in the head of the sperm where it serves in the condensation of chromatin and the stabilization of DNA. In addition to reversing the anticoagulant effects of heparin, protamine is also in intermediate- and long-acting insulin preparations where it serves to slow insulin absorption. Reversal of anticoagulation is achieved through ionic binding of protamine to the polyanionic heparin producing a stable, however not innocuous, precipitate. The heparin-protamine complex has inhibitory effects on platelet aggregation both *in vitro* and *in vivo*, an effect not found when protamine is administered alone [116, 117]. The heparin-protamine complex may also lead to more significant and dangerous effects, notably pulmonary vasoconstrictive reactions. These reactions are infrequent after cardiac surgery but have been extensively studied as they often lead to cardiovascular collapse. This phenomenon has been reliably reproduced in sheep, causing pulmonary hypertension due to increased pulmonary vascular resistance with elevated pulmonary capillary occlusion pressure and thromboxane B_2 levels and a decreased cardiac output and stroke volume [118]. This reaction was not seen when animals received protamine in the absence of heparin and pretreatment with either a cyclooxygenase inhibitor or a hydrogen peroxide scavenger abolished the increases in pulmonary vascular resistance and thromboxane levels. Similar pulmonary vasoconstrictive reactions to heparin-protamine complex accompanied by increased thromboxane levels in porcine models, including isolated pig lung preparations perfused with an acellular dextran medium [119]. Thus these pulmonary

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vasoconstrictive reactions are independent of platelet aggregation, leukocyte sequestration, and plasma component activation. Pulmonary intravascular macrophages are the source of thromboxane in these reactions as these cells respond to the infusion of various foreign particles by releasing thromboxane and other vasoactive eicosanoids and animal species lacking these reactive macrophages do not suffer pulmonary vasoconstrictive reactions [120].

Protamine administration can also have significant adverse effects independent of the presence of heparin. Protamine infusion can produce systemic hypotension by causing histamine release through the degranulation of isolated mast cells, an effect called pharmacologic release [121]. This reaction does not depend on the formation of heparin-protamine complexes and may thus occur after isolated protamine administration. Histamine release and subsequent hypotension however depends greatly on the rate of infusion and can be avoided if the protamine is administered slowly [122]. Traditional immunoglobulin-E-mediated anaphylactic allergic reaction to protamine is also well described and has been classically attributed to patients with insulin-dependent diabetes receiving neutral protamine Hagedorn-type insulin, patients with fish allergy, and patients with previous vasectomy on the basis of prior sensitization. However, anaphylactic reactions to protamine are rare and have not been shown to be more frequent in patients with the described risk factors.

Prothrombotic Factors

The initial prothrombotic factor in any CPB circuit is the circuit itself. As the heparinized blood comes into contact with the biomaterial, selected plasma proteins

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are adsorbed onto the surface to form a protein monolayer [123]. Which proteins are adsorbed and in what quantities depend on their bulk concentrations in plasma and the intrinsic surface activity of the biomaterial. These factors also determine the mosaic of the protein monolayer [124]. The proteins are densely-packed on the biomaterial surface, irreversibly bound, and immobile and are found in concentrations two to three orders of magnitude higher than in plasma [125]. Notably, the protein topography of the monolayer may not be uniform across the entirety of the biomaterial surface [124]. Moreover, the surface concentration of the adsorbed proteins may vary with time while on CPB or with the duration of CPB [126]. To further complicate the picture, adsorbed proteins may undergo minor conformational changes as a result of binding to the biomaterial, creating amino acid layouts that may interact with certain blood cells such as platelets and leukocytes or bulk plasma proteins such as the complement protein C3 and factor XII [127, 128]. Specifically, conformational changes in adsorbed factor XII and fibrinogen lead to activation of the contact pathway and platelet adhesion, respectively [128]. The contact system includes four plasma proteins: factor XII, prekallikrein, HMWK, and C-1 inhibitor. Factor XII that has been activated through adsorption with the subsequent conformational change combines with HMWK to activate the intrinsic coagulation pathway. This pathway plays a small role in thrombin generation in extracorporeal perfusion applications but also contributes to complement, platelet, and neutrophil activation [129, 130]. In contrast, the extrinsic coagulation pathway is the major source of thrombin generation during CPB and clinical cardiac surgery [131]. Activation of this pathway is principally

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through exposure of blood to tissue factor by direct contact in the wound or aspiration of wound blood into the CPB circuit [132]. Tissue factor activates factor VII to form factor VIIa/tissue factor complex, which subsequently activates factors IX and X (figure 3). Both pathways lead to the production of two different forms of tenase, an enzyme that converts factor X to its active form (Xa). Factor Xa then forms a prothrombinase complex with factor Va and cleaves prothrombin into thrombin, the active enzyme, and prothrombin fragment, F1.2 [133]. Thrombin itself cleaves soluble fibrinogen into the insoluble strands of fibrin and activates factor XIII which cross-links fibrin into the form of the mesh underlying the hemostatic clot. In addition, thrombin also activates platelets and stimulates the production of tissue plasminogen activator (t-PA) by endothelial cells, while simultaneously activating factors V and XI in positive-feedback loops [133].

Generation of Thrombin

Contact with the wound	Contact with synthetic surfaces
	$FXII + PK + HMWK \rightarrow FXIIa$
	$FXII_a + HMWK + FXI \rightarrow FXIa$
Extrinsic Pathway	**Intrinsic Pathway**

Step I:

Formation of FVII a | Formation of FXIa
1) Cellular TF + FVII + PL + Ca^{++} | 1) Thrombin + FXI
2) Plasma TF + Mono+ FVII + Ca^{++} |

Step II:

| **Formation of FIXa** |

1) (FVIIa – TF + FIX + PL) + FX→ FXa | 1) FXIa + FIX + Ca^{++} + PL → FIXa
2) FXa + FIX + Ca$^+$ + PL → FIXa |

Step III:

| **Formation of Tenase** |

1) (FVIIIa – TF + Ca^{++} + PL) | 1) FVIIIa + FIXa + FX + Ca^{++} + PL
 (Extrinsic Tenase) | (Intrinsic Tenase)

Step IV:

| **Formation of FXa** |

1) Extrinsic Tenase + FX → FXa | 1) Intrinsic Tenase + FX → FXa

Step V:

| **Formation of Prothrombinase** |

1) FXa + FVa + PL + Ca^{++}

Step VI:

| **Formation of Thrombin** |

$$\text{Prothrombin} \xrightarrow{\text{Prothrombinase}} \text{Thrombin} + \text{F1.2}$$

Figure 3: Steps in the generation of thrombin in the wound and in the CPB circuit via the extrinsic, intrinsic, and common coagulation pathways. (Reproduced with permission from the author) [3]

Platelets also participate in the thrombotic response to CPB. They are stimulated by thrombin, contact with non-endothelialized surfaces, heparin, and platelet-activating factor. Thrombin-induced stimulation is through a specific receptor [134]. In response, platelets express cellular receptors and release granule contents, releasing substances such as thromboxane A_2, platelet factor 4, beta-thromboglobulin, P-selectin, and serotonin [133]. Through their glycoprotein IIb/IIIa receptors, they bind to biomaterial surface-adsorbed fibrinogen and to each other through fibrin bridges [135]. Also, P-selectin bound to platelets simultaneously binds the platelets to monocytes and neutrophils, forming aggregates [136].

Fibrinolysis and Coagulopathy

A number of elements contribute to peri-CPB coagulopathy. Decrease in platelet number during CPB is the result of dilution, adhesion, aggregation, destruction, and consumption. Dilution is due to the use of a priming solution for the perfusion circuit that is devoid of clotting factors and platelets and that is often entirely bloodless. Dilution alone often leads to a decrease of 25-35% in coagulation factors and platelets [137]. CPB also activates large numbers of platelets, leading to adhesion to the exposed subendothelial surface or to the bypass circuit itself and to platelet aggregation. Aggregation may be with other platelets or to circulating neutrophils and monocytes [138]. Some platelets detach from the surface they have adhered to but leave behind the activated receptors, forming nonfunctional platelet microparticles [139].

Increased release of the fibrinolysis-activating enzyme t-PA occurs early after the initiation of CPB and is up to six-fold within the first five minutes [140]. As endothelial cells are the principle producers of t-PA, this marked increase in t-PA secretion is a sign of widespread endothelial cell activation [141]. PAI-1 is the main antagonist of t-PA and serum levels of this enzyme do not increase until the end of CPB, but increases 15-fold by two hours post-CPB, reducing effective t-PA activity [140]. As a result of the above, the production of plasmin, the enzyme ultimately responsible for fibrin cleavage, is markedly increased with resultant hyperfibrinolysis [142]. Plasmin generation is increased over 100-fold minutes after the initiation of CPB, which is accompanied immediately by significant fibrinolysis as demonstrated by a 200-fold increase D-dimer generation, a fibrin degradation product and marker of fibrinolysis. Even when enzyme activities attain equilibrium during CPB, in the absence of antifibrinolytic medication, the rate of D-dimer generation was similar to that of fibrin formation, signaling that fibrinolysis occurs at a rate similar to that of fibrin formation. Coagulation and fibrin formation are also hindered in the face of hypothermia, yet fibrinolysis appears to be unaffected by hypothermia as low as 33°C. Conversely, platelet activation and coagulation enzymes which are both impaired at this and less severe degrees of hypothermia [143]. Given that approximately demonstrated 35% of total fibrin generated after CPB is soluble, it would appear that this fibrin is dysregulated and not firmly bound to sites of bleeding, leading to coagulopathy at operative sites and inappropriate fibrin deposition at remotes locations [144].

Inflammatory Effects

As previously mentioned, a potent acute inflammatory response occurs in conjunction with the thrombotic reaction seen with the initiation of CPB. In fact, there is significant overlap and interaction in the effects of the cells and substances responsible for thrombosis and those responsible for inflammation. Neutrophils, monocytes/macrophages, endothelial cells, the complement system, and, to a lesser extent, platelets are the major players in the defense response to the CPB circuit. Inevitably, as part of the inflammatory response, there is generation of oxygen free radical species. These substances further aggravate the inflammatory response, which is summarized in figure 4.
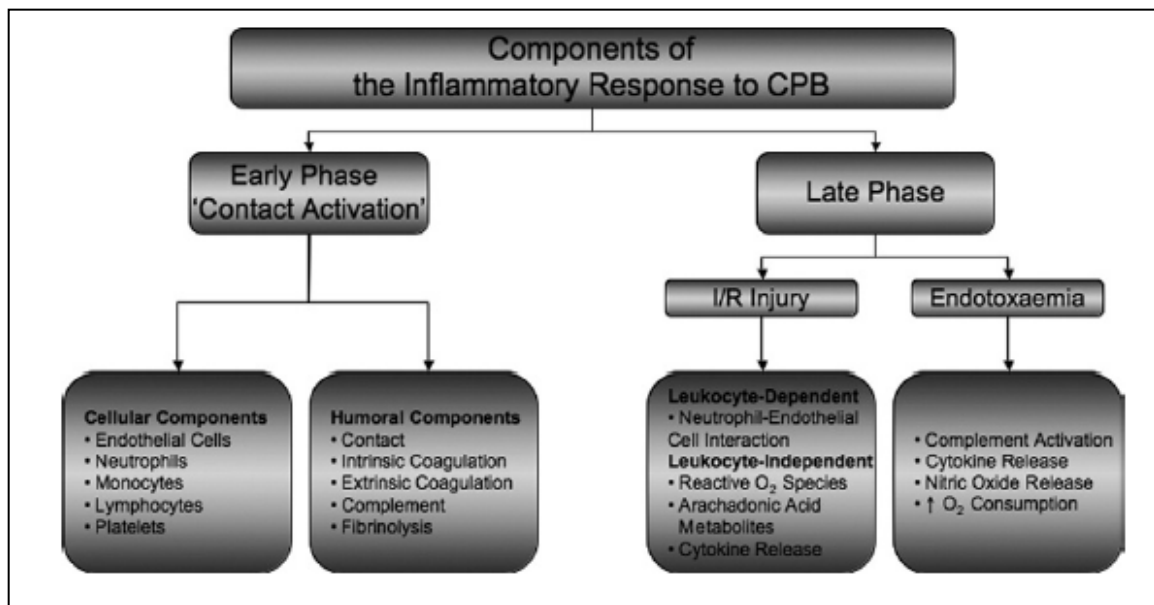
Figure 4: Summary of the inflammatory response to CPB. (Reproduced with permission from the authors) [4]

Complement System

The complement system undergoes important activation during extracorporeal perfusion during clinical cardiac surgery. It is activated upon contact with the circuit at the initation of CPB and as a result of contact with the wound [115]. Reperfusion of the ischemic heart after aortic unclamping is a second stimulus [145]. Thirdly, administration of protamine after CPB also activates the complement system [146]. Finally, C-reactive protein acts as a complement agonist and contributes to delayed inflammatory effects during the days following CPB [147]. The classical pathway is responsible for the initial activation of the complement system, in part stimulated by conformational changes in surface adsorbed C3 [115]. However, it is through the feedback loop of the alternative pathway that the majority of complement activation during CPB occurs [146]. C3a and C3b are formed through this feedback loop; C3b cleaves C5, which goes on to combine with other complement proteins to form the terminal attack complex, C5b-9 [148]. The other cleavage byproduct, C5a, along with C3a and C5b-9 are all strong anaphylotoxins with vasoactive, cytotoxic, and cell signaling properties [149]. C5a is one of the major activators of neutrophils and along with C5b-9 also stimulates expression of receptors on platelets, macrophages, and endothelial cells [150].

Neutrophils

Neutrophils are the most important cellular elements in the acute inflammatory reaction to CPB. They contain a large number of powerful proteolytic and cytotoxic enzymes as well as chemokines, cytokines, and arachidonate metabolites and store these substances in granules that can be quickly released [17]. Neutrophils also contain enzymes that produce cytotoxic reactive oxidants, free radicals, and

chloramines [151]. During extracorporeal perfusion and open-heart surgical procedures, neutrophils receive strong stimuli to release the contents of their granules into the circulation [152]. The principal stimulators are the complement and contact systems. The anaphylotoxins C3a and C5a are the principle complement system activators of neutrophils [4] while plasma kallikrein, the activated form of the contact system protein prokallikrein, stimulates neutrophil aggregation and degranulation [153]. CPB also stimulates neutrophil expression of the Mac-1 receptor, which binds fibrinogen and factor X and weakly augments thrombin formation [154]. Endothelial cells, through the production and surface expression of cell adhesion molecules that will be described later, also activate and bind neutrophils and facilitate their extravasation thus permitting a major part of their participation in the inflammatory response [155].

Monocytes

Monocyte activation during CPB is also significant but occurs more slowly than neutrophil and complement activation [4]. Some authors have proposed that monocytes are activated by complement and endotoxin [156] while others proposed additional monocyte stimulants such as contact with the CPB circuit, endotoxin, and circulating tissue factor [4]. Once activated, these cells secrete a variety of pro- and anti-inflammatory cytokines, including the interleukins IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, and IL-12. Other than the inhibitory IL-12, these cytokines activate neutrophils and endothelial cells and promote further inflammation [157]. The significance of another important pro-inflammatory cytokine, the tumor necrosis factor (TNF), in the inflammatory response to CPB is unclear in the

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literature, with discordant reports on whether TNF levels increase. Inflammatory injury to the lung and organs may depend on monocyte activation and extravasation into organ parenchyma and subsequent monocyte differentiation into macrophages [156]. Monocytes also play a role in the prothrombotic _milieu_ during CPB with significant upregulation of tissue factor expression [158] and with the formation of platelet-monocyte conjugates [159].

Lymphocytes

Lymphocytes play a minor role in the inflammatory reaction after CPB compared to the other leukocytes discussed. In fact, CPB appears to be inhibitory to lymphocytes, in contrast with the generally stimulatory effect it has on other immune cells. Blood lymphocyte concentrations fall during CPB and the effect is seen in nearly all lymphocyte subpopulations, including B-lymphocytes, natural killer cells, T-helper cells, and T-suppressor lymphocytes [160]. It is only after a week that lymphocyte numbers return to normal. Lymphocytes are also qualitatively impaired after CPB, with decreased responses to mitogens and antigen recognition [161]. Lymphocyte function also takes one week to return to normal.

Platelets

Platelet interaction with circulating leukocytes was briefly described earlier. CPB leads to significant decreases in platelet number but also significant platelet activation. Platelets contribute to the inflammatory process in a few ways. They form conjugates with monocytes by expressing the adhesion molecule P-selectin that binds to P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes [162].

Activated platelets use this P-selectin/PSGL-1 adhesion pathway to stimulate their partner monocytes to secrete proinflammatory cytokines IL-1, IL-8, and MCP-1 [163]. This interaction involving P-selectin also induces fibrin deposition and tissue factor expression by monocytes [164, 165]. Platelet interaction with endothelial cells also contribute to inflammation. Platelets express a ligand, CD40L, for an endothelial cell adhesion molecule, CD40. This ligand is structurally related to TNF and through its binding, endothelial cells are stimulated to secrete the chemokines IL-8 and MCP-1 and to express adhesion molecules [166]. This process generates signals for the recruitment and extravasation of leukocytes at the site of injury.

Endothelial Cells

As described in length above, under normal conditions endothelial cells provide a non-thrombogenic inner lining to the vasculature, participating in the regulation of vascular tone in response to physiologic stimuli and in hemostasis when necessary. However, as with the other cellular blood elements described, they undergo stimulation during CPB and open heart surgery and their function and their phenotype are altered. This process is known as "endothelial cell activation." These agonists include hypoxia, hemodynamic shear stress, surgical manipulation, thrombin, C5a, IL-1, and TNF [3, 167-169]. Less important agonists include endotoxin, histamine, and lymphocyte-derived interferon-γ while chemokines have relatively little effect on endothelial cells [3]. Endothelial cells activation can be differentiated into two types [169]. In the first, the restoration of blood flow to ischemic tissues induces within seconds to minutes the expression of proteins already stored within endothelial cells that promote leukocyte-endothelial cell

interactions and coagulation. The second type is in response to cytokines and leads to transcription of specific genes with eventual translation into protein over the course of several hours.

Regardless of the type of activation, the eventual result is the same. Endothelial cell activation leads to an increase in surface expression of E-selectin, endothelial leukocytes adhesion molecule (ELAM), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule (VCAM-1), all mediators of leukocytes recruitment [167]. These molecules bind neutrophils and monocytes to the endothelium and instigate their translocation to the interstitium. While E-selectin is selectively expressed by endothelium, ICAM-1 and VCAM-1 can be expressed by endothelium, leukocytes, and a variety of other cell types and are in fact members of the immunoglobulin superfamily [170]. ICAM-1 contains five immunoglobulin-like domains and is constitutively expressed at low levels on the resting endothelium with a marked increase in response to cytokines such as IL-1 and TNF. Conversely, VCAM-1 is not expressed on resting endothelial cells and its expression is induced by IL-1, IL-4, and TNF [171]. It too has immunoglobulin-like regions (seven of them) in its N-terminal domain. Other molecules that are secreted by activated endothelial cells are the proinflammatory cytokines IL-1, IL-6, IL-8, MCP-1, and PAF [172].

Endothelial cell activation also has effects on vascular tone. Stimulation by IL-1 and TNF leads to increased production of prostacyclin through stimulation of cyclooxygenase and of NO by NOS [173, 174]. These vasodilators reduce shear stress and increase vascular permeability, enhancing leukocyte adhesion and endothelial transmigration. Simultaneously, activated endothelial cells also produce

the vasoconstrictor endothelin-1 and inactivate certain vasoactive substances including histamine, bradykinin, and norepinephrine [175]. While endothelin-1 levels peak several hours after CPB [176], prostacyclin concentrations reach their acme early after the initiation of CPB and decrease subsequently [177].

Pulmonary Injury

Cardiopulmonary bypass has significant effects on pulmonary function, specifically on lung mechanics, gas exchange, and inherent lung metabolism. In fact, simple general anesthesia and mechanical ventilation themselves have important negative repercussions on respiratory physiology. Resting muscle tone and hypoxic vasoconstrictive reflexes are both reduced by general anesthesia and muscular paralysis and lead not only to atelectasis but also worsening ventilation-perfusion mismatch [178]. Furthermore, respiratory work is increased during anesthesia as a combined result of reduced lung compliance and increased airway resistance. Considerations specific to cardiac surgery include left lower lobe atelectasis post-CPB in 60-70% of cases due to the weight of the flaccid heart compressing lung parenchyma and frequent entry into the left pleural cavity with dissection of the left internal mammary artery [179]. These changes can lead to hypoxemia, which varies from clinically insignificant to severe. CPB may aggravate these changes in pulmonary mechanics but the effects appear to be less important than those of pleurotomy and lung compression [180, 181].

While it would seem that the effects of CPB on respiratory mechanics add relatively little to that of the rest of the intervention, CPB also produces a type of lung injury

that, in its extreme, resembles the acute respiratory distress syndrome (ARDS).

Even patients with uneventful postoperative courses after cardiac surgery with CPB

demonstrate injury and swelling of endothelial cells and pneumocytes on

examination of lung biopsy specimens [182]. Notably, during CPB pulmonary

arterial blood flow is usually minimal or absent as the systemic venous return that

would normally travel through the right heart cavities to the lungs is instead

diverted to the pump. Consequently, the bronchial arteries are the main blood

supply to the lungs during full CPB. A third source of oxygenation for the lungs is

alveolar ventilation which is also interrupted during CPB in order to improve

operative exposure. While some oxygenation is obviously maintained, the lungs

experience a certain degree of ischemia nonetheless. When CPB is subsequently

weaned and lung perfusion re-established, the lungs experience ischemia-

reperfusion (IR) injury. This CPB-induced injury strongly resembles the insult

experienced by lungs during transplantation and as such a substantial proportion of

the literature on lung IR stems from lung transplantation studies. This response is

shown schematically in figure 5.
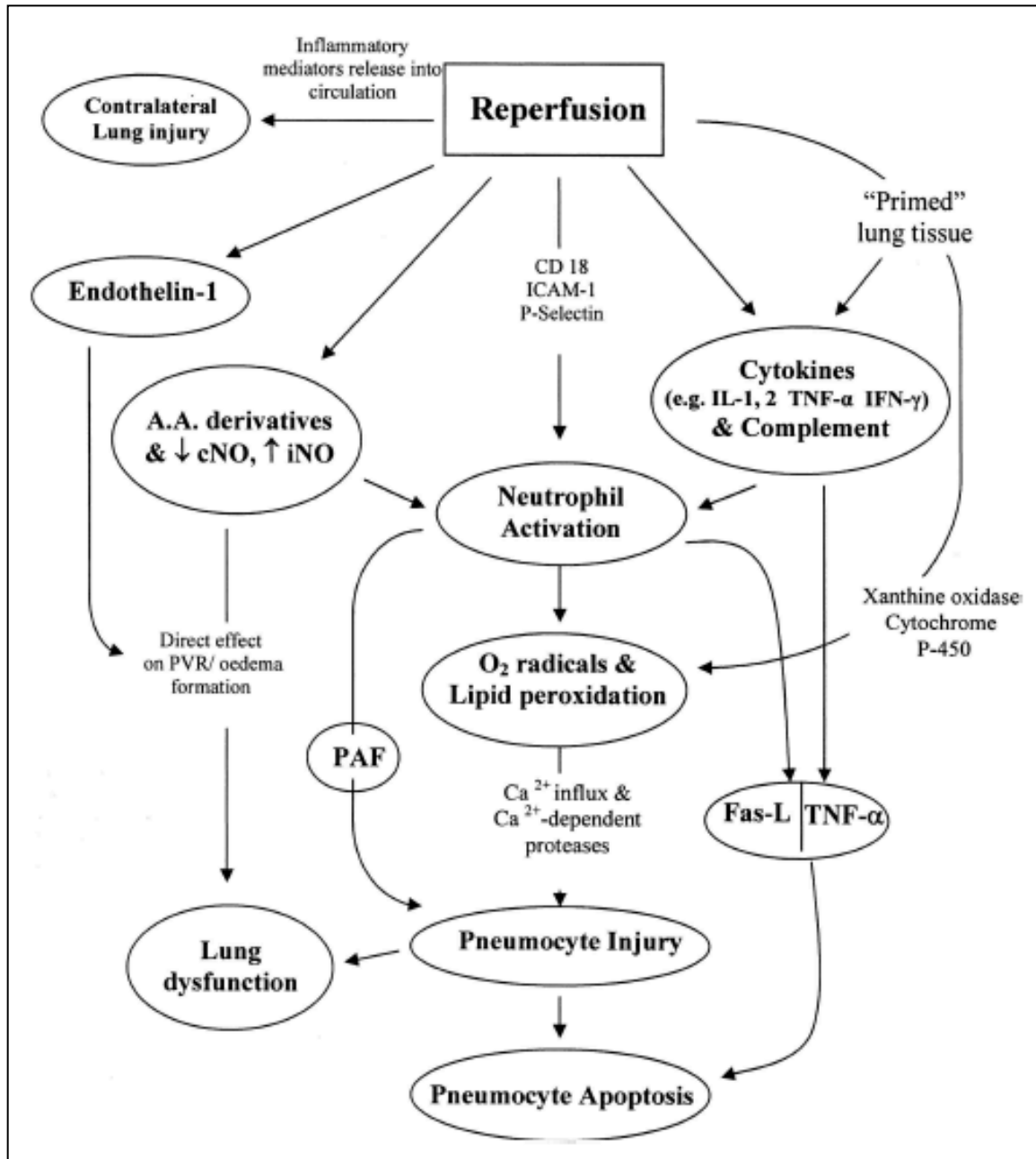
Figure 5: Inflammatory response following reperfusion of ischemic lung.

(Reproduced with permission from the authors) [5]

The initial cellular response consists of endothelial cell rounding and contraction,

rather than cell lysis, as demonstrated in an in vitro model [183]. These changes are

essentially those of endothelial cell activation, as described earlier, with increased

surface expression of adhesion molecules and leukocyte recruitment. Among these is ICAM-1, which is upregulated in pulmonary vessels during CPB [184, 185]. The cytokine IL-8 is significantly increased in lung tissue and the systemic circulation after lung IR [186, 187]. This cytokine stimulates pulmonary neutrophil migration, activation, and degranulation. Increased binding and activation of NF-κB appears to mediate IL-8 gene induction in hypoxic endothelial cells [187]. In the model above, the authors were able to replicate the changes in the endothelial cells by administering recombinant TNF. The overall result is pulmonary neutrophil infiltration and increased vascular permeability. Other changes seen in animal lung biopsy specimens obtained following lung IR injury included alveolar capillary interstitial edema, hyaline membranization of alveolar ducts, polymorphonuclear cell (PMN) infiltration of pulmonary vessels, and detachment of endothelial cells and type I pneumocytes from the basement membrane [5]. Elevated levels of lipoxygenase metabolites including thromboxane B2 (TXB_2), 15-hydroxyeicosatetraenoic acid (15-HETE), 5-hydroperoxyeicosatetraenoic acid (HPETE), prostaglandin E2 (PGE2), leukotrienes (LT) B4/C4/D4, 6-keto-prostaglandin F1 (PGF1)-α, and 12-hydroxyheptadecatrienoic acid (HHT) have been found in blood and bronchoalveolar lavage (BAL) samples following reperfusion in animal lung IR and transplant models [5]. These findings are associated with significant increases in pulmonary vasoconstriction, pulmonary neutrophil sequestration, microvascular permeability, and pulmonary edema. Increased plasma and BAL endothelin-1 levels have also been detected post-reperfusion in animal lung IR and transplant models, with many of the same consequences, notably

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increased pulmonary vascular resistance and pulmonary artery pressures (PAP). Additionally, endothelin-1 may have synergistic effects with PAF, whose release from activated PMNs, endothelial cells, and macrophages is upregulated by lung IR injury. These combine to cause a reduced cardiac index, poor oxygen and carbon dioxide tensions, decreased lung compliance, along with elevated pulmonary vascular resistance and arterial-alveolar oxygen difference and worsened pulmonary edema [5, 188]. An additional vasoconstrictor that has been shown to be produced by endothelial cells in response to hypoxia is platelet-derived growth factor [189].

Lung IR is also associated with the generation of oxygen free radicals occurring during IR of other organ systems. Reactive oxygen radicals are produced as a result of hypoxia-induced activation of xanthine oxidase and the mitochondrial cytochrome P-450 system [190]. These oxygen free radicals can induce direct endothelial cell damage by increasing intracellular calcium and activating Ca^{2+}-dependent proteases, ultimately leading to cell death [191]. Beyond causing cell death, oxygen free radicals have been shown *in vitro* to contribute to endothelial dysfunction, with decreased EDRF production and consequent decreased cGMP accumulation in vascular smooth muscle cells [192].

As described above, multiple pulmonary vasoconstrictive influences have been demonstrated in lungs following IR injury. However in addition to this release of vasoconstrictors, there is also a decrease in basal vasodilator activity. Lung NOS expression and tissue cGMP levels were both significantly reduced after reperfusion with consequently increased mean pulmonary artery pressure in a lung IR model

[185]. Similarly, decreased pulmonary NO production associated with increased pulmonary vascular resistance was found in piglets having undergone standard CPB, and was largely reduced in animals pretreated with antioxidants [193]. This phenomenon is due to post-CPB pulmonary endothelial dysfunction, which has been demonstrated *in vivo* in a piglet model of CPB with deep hypothermic circulatory arrest (DHCA) [194]. While vascular smooth muscle function and the ability of the pulmonary endothelium to generate NO remains intact, these researchers showed a loss of ACh-mediated endothelium-dependent vasodilation. Vascular reactivity studies performed by a different group on pulmonary arteries isolated from piglets having undergone CPB *without* circulatory arrest demonstrated a similar decrease in ACh-mediated endothelium-dependent vasodilation with preserved endothelium-independent relaxation [195]. Interestingly, arteries from skeletal muscle showed no changes in contractile or relaxant properties after CPB, perhaps indicative of the relatively preserved perfusion of muscular tissue during CPB as compared to the lung. Overall, pulmonary endothelial dysfunction, with the resulting decrease in vasodilator tone, contributes significantly to the increased pulmonary vascular resistances seen post-CPB.

In the end, clinical cardiac surgery with CPB submits the lungs to a multifactorial injury which are affected by the patient's underlying cardiac and pulmonary conditions. The insults resulting from general anesthesia, mechanical ventilation, and the physical effects of the surgery itself add on to the dual injury that CPB inflicts on the lungs: inflammation and ischemia-reperfusion. The cumulative result is lungs with disturbed mechanics in addition to activated and dysfunctional

endothelium that causes increased vascular permeability with associated interstitial edema, leukocytic pulmonary infiltration, and increased pulmonary vascular resistance. These factors may obviously lead to respiratory failure but cardiac dysfunction may also ensue, with right ventricular failure as a result of increased afterload from pulmonary vasoconstriction which carries a dismal prognosis.

FOURTH CHAPTER

PULMONARY HYPERTENSION IN CARDIAC SURGERY

Pulmonary hypertension is an important problem in clinical cardiac surgery principally due to its potential negative impact on right ventricular (RV) function and postoperative outcomes. Pulmonary hypertension per se was associated with an odds ratio for mortality of 2 or more in several large studies of cardiac surgical risk factors [196, 197]. When RV failure is precipitated by pulmonary hypertension after cardiac surgery, early mortality may be as high as 20% to 44% in reported series [198, 199]. The prevalence of pulmonary hypertension associated with heart disease is difficult to estimate. In Western countries, the prevalence of pulmonary hypertension associated with congenital heart disease is estimated to range between 1.6 and 12.5 cases per million adults, primarily in patients with systemic-to-pulmonary shunts [200]. The prevalence of pulmonary hypertension in patients with acquired heart disease is even harder to quantify. Acquired disorders leading to pulmonary hypertension consist of left ventricular dysfunction, aortic and mitral valve disease, and constrictive pericardial disease. While mitral stenosis was the most frequent cause of pulmonary hypertension decades ago, it has been surpassed in the Western world by left ventricular dysfunction as the most common etiology. Patients with pulmonary hypertension due to acquired heart disease are in Group 2 (Pulmonary Hypertension with Left Heart Disease) of the Clinical Classification most recently revised at the 4th World Symposium on Pulmonary Hypertension in 2008 [201]. This group was formerly classified under pulmonary *venous* hypertension. Patients with congenital heart disease associated-pulmonary hypertension are within a subdivision of group 1, pulmonary *arterial* hypertension.

The pathophysiology of preoperative pulmonary hypertension of these two types is initially different but ultimately leads to the same type of pulmonary vascular remodeling. In congenital heart disease with systemic-to-pulmonary shunting, pulmonary blood flow is increased. Evidence suggests that the chronically increased pulmonary blood flow leads to endothelial dysfunction from the shear stress [202, 203]. Endothelial dysfunction appears to be a precursor to smooth muscle dysfunction and proliferation which leads to the histological changes in the pulmonary arteries, first characterized in pulmonary hypertension patients in 1958 [204]. These changes begin with muscularisation of the small pulmonary arterioles, then progress to medial hypertrophy with intimal proliferation. While at this stage these modifications may still be reversible, there is usually progression to irreversible intimal fibrosis and occlusion with eventual development of plexiform and chronic dilatation lesions unless the congenital shunt is corrected. In conditions leading to pulmonary venous hypertension, the first changes affect the pulmonary capillary bed [205]. Increased venous pressure leads to swelling of pulmonary capillary endothelial cells with basal laminar thickening. Interstitial edema follows and chronic exposure will lead to proliferation of fibrous connective tissue around the alveoli. Late changes in the pulmonary capillary bed include transudation of erythrocytes through ruptured membranes into the alveolar spaces, with hemosiderosis, fibrosis, and hemorrhage. The pulmonary lymphatics may also undergo marked distension in order to compensate for the increased transudation of fluid induced by the elevated pulmonary venous pressure. Changes in the arterial bed may then ensue, as described above.

Superimposed on these preoperative factors (pre-existing pulmonary hypertension, valvular disease, or left ventricular dysfunction) are a number of peri- and postoperative factors that may lead to or aggravate pulmonary hypertension, summarized in figure 6 [6]. The inflammatory reaction to CPB and the pulmonary ischemia-reperfusion injury, both described in length earlier, along with blood transfusions may exacerbate existing pulmonary hypertension. New left-sided valvular lesions or decreased left ventricular function due to technical or myocardial protection factors are a frequent cause of pulmonary hypertension. Much more rare is the catastrophic pulmonary vasoconstriction that may occur due to administration of protamine. Explained in length earlier, briefly, formation of the heparin-protamine complex occasionally leads to dramatic production of thromboxanes, which provoke severe pulmonary vasoconstriction. This reaction occurs in up to 1.8% of patients undergoing cardiac surgery and is associated with adverse hemodynamic responses ranging from minor perturbations to cardiovascular collapse [206]. Residual cardiac structural lesions may also contribute to post-CPB pulmonary hypertension. In particular, residual left-to-right shunts and left-sided stenotic lesions (especially of the mitral valve) may also aggravate pulmonary hypertension.
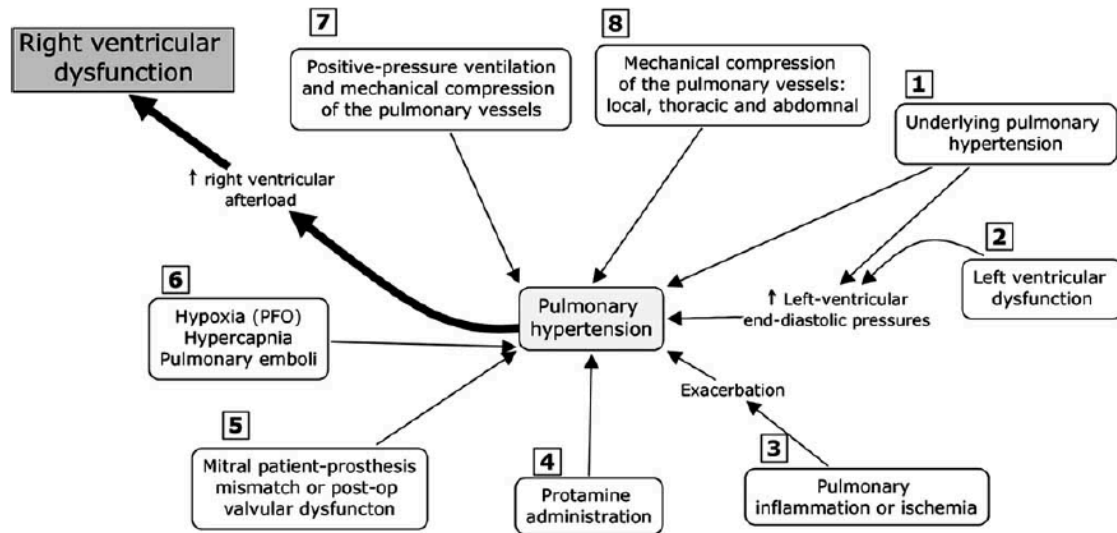
Figure 6: The most common mechanisms contributing to pulmonary hypertension in cardiac surgery. (Reproduced with permission from the authors) [6]

Perioperative factors not exclusive to cardiac surgery such as hypoxia, hypercarbia, and pulmonary embolism (rare in the immediate postoperative period in cardiac surgery) also may cause pulmonary hypertension. As will be described later, both hypoxia and hypercarbia can lead to pulmonary vasoconstriction. Other ventilatory factors may lead to fluctuations in pulmonary vascular resistance (PVR) in other ways. There is a unique U-shaped relationship between lung volumes and PVR, which is due to the mixed contribution of extra- and intraalveolar vessels [207]. PVR is minimal at functional residual capacity and is increased at volumes that are larger or smaller. Notably, hyperinflation will lead to significantly increased PVR. High ventilatory pressures, in particular positive end-expiratory pressure, can lead to compression of pulmonary capillaries in the well-ventilated lung zones, thus diverting blood flow to areas with poorer ventilation and contributing to hypoxia.

Patients suffering from pulmonary hypertension and undergoing cardiac surgery remain a challenging population to treat. Pathological changes in the pulmonary vasculature due to often long-standing structural lesions are important substrates for post-CPB pulmonary hypertension that may be aggravated by a number of perioperative factors. Understanding these points is the first step in understanding how to optimize treatment.

FIFTH CHAPTER

OPTIONS FOR TREATMENT OF POST-CARDIOPULMONARY BYPASS

PULMONARY HYPERTENSION

The treatment of post-CPB pulmonary hypertension is similar in many ways to the treatment of pulmonary hypertension in most other contexts. However, knowing that high-risk patients will be exposed to the provoking factor of CPB affords physicians the opportunity to treat prophylactically, often by administering a treatment prior to the beginning of CPB, during CPB, and/or prior to CPB weaning. Many of the treatments that are used prophylactically can also be effective to treat a pulmonary hypertension if it has occurred. The ultimate goal is to avoid/treat acute right ventricular failure that may arise as a consequence of the increased pulmonary vascular resistance (RV afterload) and that could lead to cardiogenic shock and subsequent multi-organ failure. A number of non-pharmacologic therapies based on our understanding of pulmonary vascular physiology have been used to reduce PVR while right ventricular function is otherwise optimized. Also, beginning in the early 1990s, targeted pharmacologic therapies for pulmonary hypertension have become increasingly available and numerous agents including prostaglandin analogues, inhaled nitric oxide, phosphodiesterase inhibitors, and endothelin-receptor antagonists, as well as specific agents are used to prevent CPB-associated pulmonary hypertension.

Non-pharmacologic Therapies

Non-pharmacologic treatment of pulmonary hypertension/vasoconstriction takes advantages of the characteristics by which the pulmonary vasculature differs from that of the systemic circulation. Changes in oxygenation and ventilation can have important effects on pulmonary vascular tone, unlike with the systemic circulation. The pulmonary hypoxic vasoconstrictor response, known as the Euler-Liljestrand

reflex, is a normal adaptive mechanism whereby small pulmonary arteries and arterioles will constrict in response to the low oxygen tension of gas that diffuses directly from surrounding respiratory bronchioles, alveolar ducts, and alveoli. This mechanisms limits blood flow to hypoxic (presumably under-ventilated) lung zones and serves to reduce ventilation-perfusion mismatch and is beneficial in normal human physiology. However, chronic hypoxemia can lead to vascular remodeling and sustained elevation of PAP; in its extreme form, vasodilation in response to oxygen no longer occurs. The constrictive response of VSMC of pulmonary arteries to hypoxia begins within seconds. Hypoxia-induced changes in the membrane redox status leads to VSMC depolarization and inhibition of potassium currents through the Kv1.5 channels [208]. Increased calcium ion entry into the VSMC through L-type Ca^{2+} channels and Ca^{2+} liberation from the sarcoplasmic reticulum also contribute to pulmonary hypoxic vasoconstriction. While *alveolar* oxygen tension is the major determinant of pulmonary vascular tone, reduced oxygen tension in the mixed venous blood flowing through the pulmonary arterioles also leads to pulmonary vasoconstriction [209].

Acidosis also causes an increase in pulmonary vascular tone, and the vasoconstrictive effect is synergistic with hypoxia [208]. While it is commonly known that increased arterial pCO2 can lead to pulmonary vasoconstriction, CO_2 per se has little effect on the vascular tone. Rather, it is via the induced increase in H^+ ion concentration that the vasoconstriction is induced [208]. Maintenance of a slightly alkaline pH (7.45-7.50) through a combination of ventilatory adjustments and infusion of exogenous base may be helpful to reduce pulmonary vasoconstriction.

Accordingly, pulmonary vascular tone may be manipulated in a perioperative setting in patients who are mechanically ventilated by adjustment of ventilator settings. The fraction of inspired oxygen may be increased up to 1.0 on the ventilator while the alveolar ventilation may be adjusted in order to maintain pCO_2 at or slightly below the lower limit of normal. While positive-pressure ventilation has no direct effect on pulmonary vascular tone, high ventilatory pressure exerts inward radial force on the intrapulmonary vasculature and can increase the effective PAP seen by the RV. While it is desirable to minimize positive end-expiratory pressure (PEEP) in the ventilator circuit as a part of reduction of ventilation pressures, PVR is at its lowest when pulmonary function residual capacity (FRC) is normal. Hence, the PEEP level must be adjusted to be the lowest possible while maintaining normal or near-normal FRC.

General Cardiovascular Management

Other important aspects of the management of RV failure include maintenance of coronary perfusion through preservation of systemic blood pressure, optimization of RV preload and contractility through fluid infusion/diuretics and inotropes, and elimination of any other factors that may influence RV afterload and dynamics (such as LV failure which causes both pulmonary venous hypertension and rightward septal shift).

Inhaled Nitric Oxide

The capacity of inhaled NO (inhNO) to decrease pulmonary vasoconstriction was demonstrated in 1991 both in patients with severe pulmonary hypertension and in

patients suffering from cardiac disease but with normal PVR with no decrease in systemic vascular resistance (SVR) in any case [210]. inhNO was later shown to reduce elevations in PAP due to hypoxic vasoconstriction in healthy volunteers breathing 12% oxygen [211]. While both of these studies were in adults, the only currently accepted indication for inhNO by either the U.S. Food & Drug Administration or Health Canada is in term or near-term neonates with hypoxic respiratory failure associated with pulmonary hypertension, where it has shown to improve oxygenation and reduce the usage of extracorporeal membrane oxygenation but no difference in mortality in a few randomized trials [212, 213]. inhNO is most frequently used off-label as a vasodilator during pulmonary vasoreactivity testing as part of workups and in the heart transplant population, however there is increasing literature concerning the use of inhNO in acutely ill patients with severe pulmonary hypertension or ARDS with refractory hypoxemia. The rationale underlying inhalation of NO in preference to intravenous administration of NO donors such as nitroglycerin or sodium nitroprusside is that inhNO will distribute to the alveoli and alveolar ducts and will act locally as a vasodilator on the surrounding pulmonary arterioles, while the short half-life of NO will prevents its distribution into the systemic circulation and the undesirable consequent decrease in systemic vascular resistance. The localized action of inhNO is also the source of its theoretical effectiveness in ARDS. While intravenous NO donors can worsen hypoxemia by abolishing hypoxic vasoconstriction, only the arterioles of ventilated portions of the lungs will be exposed to the vasodilator and thus the better ventilated parts of the lung will also receive better perfusion,

improving the ventilation-perfusion matching. The doses required for improving hypoxemia are lower than those for decreasing PVR. Toxicity is relatively minimal and animals studies have shown safe use of inhNO at 40 parts per million [89] for up to six months. However, NO reacts with hemoglobin to form methemoglobin and there may be accumulation in a pediatric population, while adults without methemoglobin reductase deficiency usually do encounter this problem [214].

Inhaled NO in Cardiac Surgery

Shown to decrease PVR, inhNO began to be used in clinical cardiac surgery. inhNO at doses varying from 2 to 10 ppm was administered to infants undergoing corrective surgery for congenital defects with left-to-right shunts at risk of postoperative pulmonary crisis [215]. A better response was not surprisingly seen in patients who had high pulmonary to systemic artery pressure ratio (greater than 0.5). These patients demonstrated a decrease in mean pulmonary vascular resistance index by 37% to 42%, accompanied by only a 10% fall in the systemic vascular resistance index but a 14% to 16% rise in mean cardiac index. No patient suffered from NO toxicity in this study. Several years later, inhNO was studied in critically-ill adults suffering from acute right heart failure associated with pulmonary hypertension of multiple etiologies, including post-cardiac surgery [216]. Fourteen of 26 patients in this study (54%) showed improvement with inhNO of over 20% in cardiac output and/or decrease in PVR with essentially no change in SVR. The mean concentration of inhNO required to achieve these effects was 35 ppm, and 85% of patients exhibiting a substantial improvement in hemodynamics did so at a concentration of inhNO of less than or equal to 40 ppm. Use of inhNO in patients with preoperative

pulmonary hypertension undergoing cardiac surgery has since become relatively commonplace and has shown benefit compared to standard therapy with oxygen in several studies. Patients with significant mitral valve stenosis and severe pulmonary hypertension were randomized to inhNO at 10 ppm or oxygen according to standard protocols in order to maintain arterial oxygen saturation of at least 95% [217]. Patients receiving inhNO had significantly greater decreases in PVR compared to the oxygen group and greater increases in cardiac output, which translated into lesser need for systemic vasoactive drugs and a shorter intensive care unit stay. Benefits of inhNO was also shown in infants undergoing cardiac surgery for correction congenital heart lesions with high pulmonary flow, pressure, or both and objective preoperative evidence of pulmonary hypertension [218]. These children were at risk of postoperative pulmonary hypertensive crises (PHTC, defined as an increase in the pulmonary to systemic pressure ratio to over 0.75) and were randomized to inhNO or placebo gas. Infants receiving inhNO continuously at 10 ppm until extubation suffered from significantly fewer PHTC and were quicker to reach extubation criteria than their counterparts receiving placebo nitrogen. Finally, in heart transplant recipients with preoperative pulmonary hypertension, another important group at risk of RV failure due to pulmonary hypertension, also from inhNO. Administration of inhNO at 20 ppm lead to reduction in PVR, mean PAP, and RV stroke work index without changes in systemic pressure, cardiac indices, left atrial pressure, or SVR [219]. All in all, significant evidence exists for the use of inhNO at doses between 10 and 40 ppm for reduction of PVR after cardiac surgery. The principal limitation to inhNO use remains its high cost.

Prostanoids and Prostanoid Analogs

Several prostanoid/prostanoid analogues exist for use in the treatment of pulmonary hypertension, all of which act through the receptor for prostacyclin. Epoprostenol, marketed under the trade name Flolan (GlaxoSmithKline, London, United Kingdom), is a synthetically-generated prostacyclin. Iloprost (Actelion Pharmaceuticals, San Francisco, California) and treprostinil (United Therapeutics, Silver Spring, Maryland) are prostacyclin analogues. As described earlier, activation of prostacyclin receptors on VSMC leads to activation of adenylate cyclase, increased intracellular cAMP and calcium levels with consequent inhibition of the cellular contractile machinery. Prostacyclin receptors also stimulate ATP-sensitive potassium channels that lead to hyperpolarization of the cell membrane and hence vasodilatation.

Epoprostenol is the best studied of the prostanoid therapies. For long-term therapy, it is administered intravenously through a permanently implanted central venous catheter using a portable infusion pump and improves hemodynamics, functional capacity, and survival in ambulatory patients with idiopathic pulmonary arterial hypertension [220] [221, 222]. In ambulatory patients with other non-idiopathic types of World Health Organization (WHO) group I PAH, including patients with congenital heart diseased-related PAH, studies similarly demonstrated improved hemodynamics and functional capacity [223, 224]. Treprostinil has also shown efficacy in improving functional capacity and hemodynamics when used in long term treatment with either continuous intravenous and subcutaneous administration [225, 226]. Successful transition from intravenous epoprostenol to

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subcutaneous treprostinil has been reported and may be desirable in order to reduce the more significant adverse effects reported with the former [227]. These complications are all related to the need for central venous delivery of the agent and include thrombosis, pump malfunction, and catheter infection. More common adverse effects include jaw pain, diarrhea, and arthralgias. Inhaled administration also avoids the complications related to central venous access and is possible with iloprost and recently with treprostinil. Inhaled iloprost improved WHO class and exercise tolerance versus placebo when used for 12 weeks; however, administration is required six to nine times per day [228]. Inhaled treprostinil requires less frequent administration and produced a good hemodynamic response in pilot studies [229].

Use in Cardiac Surgery

Short-term use of prostacyclin and its analogues as in the context of cardiac surgery necessitates slightly different constraints than the ambulatory long-term treatment of PAH. The rationale is similar to that supporting the preference for inhNO over intravenous nitrates. Compared to the ambulatory setting, inhaled administration is simplified in the intubated patient and continuous inhalation is possible. Its short half-life of 5 minutes and rapid hydrolysis at acid or physiologic pH to 6-keto-prostaglandin $F_1\alpha$ prevents the systemic hypotension that may be seen with intravenous therapy [230]. Experimentally, treatment with prostacyclin/prostacyclin analogues before CPB has been shown to prevent post-CPB increases in pulmonary artery pressure post-CPB as well as improving pulmonary endothelial dysfunction. A porcine study from our laboratory showed

that these benefits of inhaled prostacyclin were related to increased intracellular cAMP in pulmonary VSMC [231]. In human trials and in clinical practice, either inhaled epoprostenol or iloprost is used. In one study, 126 patients undergoing cardiothoracic surgery and suffering from pulmonary hypertension, severe hypoxemia, or right heart failure received inhaled epoprostenol [232]. The majority of these patients underwent cardiac surgery (62%), while 34% underwent lung transplantation and the remaining 4% underwent another thoracic surgical procedure. Inhaled epoprostenol in patients with pulmonary hypertension decreased mean PAP by 16.7% ($p<0.001$), PAP/mean arterial pressure (MAP) ratio by 18.4% ($p<0.001$), and PVR by 31% ($p=0.005$) versus baseline values in patients with pulmonary hypertension, with no significant change in MAP and a trend towards improved cardiac output. In patients with right ventricular dysfunction, cardiac output improved by 15.8% ($p=0.036$), PAP decreased by 14.7% ($p=0.007$), and PAP/MAP ratio decreased by 18.6% ($p=0.002$), again with no significant change in MAP. No patients in this study suffered from side effects from administration of inhaled epoprostenol.

Prostanoids vs. inhaled NO

With time, inhaled epoprostenol and iloprost have come to be looked upon as alternatives to inhNO. All three agents function as selective pulmonary vasodilators, however inhNO requires a costly proprietary drug delivery system as well as monitoring of toxic metabolites. Researchers have consequently sought to determine if inhNO provides greater efficacy compared to prostanoid agents that would justify the added cost. A randomized study comparing inhaled iloprost to

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inhNO (at 10 ppm) was performed in infants undergoing corrective cardiac surgery for left-to-right shunts with high pulmonary blood flow and demonstrated pulmonary hypertension preoperatively and at the conclusion of CPB [233]. There was no difference in mean PAP, PAP/MAP ratio, PVR, and cardiac output nor in the frequency of postoperative pulmonary hypertensive crises between the two groups although both treatments did improve hemodynamics. A different study examined adults with pulmonary hypertension waiting to undergo a variety of cardiac surgical procedures (excluding transplantation and ventricular assist device implantation) [234]. Patients were randomized to receive either inhaled iloprost or inhNO (at 20 ppm) after weaning from CPB. Both medications produced significant improvements in mean PAP and PVR, however iloprost caused significantly greater reductions in PVR (p=0.013) and mean PAP (p=0.0006) and a significantly greater increase in CO (p=0.002) than inhNO. Interestingly, both groups of patients also exhibited a significant decrease in SVR after the initiation of treatment (p=0.0001), which was statistically significantly greater in the iloprost group 30 minutes after the start of therapy (p=0.016) but not at 90 minutes (p>0.2). The authors speculated that the decrease in SVR could be partly due to the confounder of onset of post-CPB vasoplegia in addition to spillover of iloprost from the pulmonary circulation into the systemic circulation, due to its relatively longer half-life compared to NO (30 minutes versus several seconds). Other advantages mentioned include easier administration/availability than inhNO and lesser cost. Another study randomized patients to inhaled epoprostenol, inhNO, or control [235]. All patients suffered from severe mitral valve stenosis and pulmonary hypertension with high PVR and

underwent mitral valve repair or replacement. Mean PAP and PVR both significantly decreased by 42.1-42.9% and 52.3-58.3% versus baseline respectively in both the inhNO and epoprostenol groups, which was statistically significant compared to both baseline ($p<0.05$) and versus the control group ($p<0.05$). Right ventricular ejection fraction (RVEF) progressively improved after treatment with both inhNO and epoprostenol and at the time of chest closure was significantly greater in these groups than at baseline and than in the control group (45-54% increase in RVEF versus baseline, $p<0.05$ versus baseline and versus control group). Patients in the inhNO and epoprostenol groups also had less need for inotropes and vasopressors than those in the control group, had shorter intubation times, and shorter intensive care unit stays (all $p<0.05$) but no difference in in-hospital mortality. While it remains unclear whether the hemodynamic benefits of inhaled prostacyclin or its analogues outweigh those of inhNO in the postoperative setting, there are definite advantages in terms of ease of administration, treatment cost, and absence of toxic metabolites.

Endothelin Receptor Antagonists

The first description in the literature of endothelin-1 receptor antagonists (ERA) was in the early 1990s, with the earliest description of bosentan (Actelion Pharmaceuticals, San Francisco, California), currently the best studied ERA, coming in 1994 [236]. Bosentan is a non-selective ERA, with inhibitory effects on both ET_A and ET_B receptors. Since its development, several new *selective* ERA were created, targeting the ET_A receptor. While most ERAs have been studied for use against the vasoconstrictive properties of endothelin-1, atrasentan (Abbott Laboratories,

Abbott Park, Illinois) and zibotentan (AstraZeneca, Macclesfield, UK) are selective

ERAs that were studied for use against cancer. The majority of experience with

ERAs has been with three specific agents and is in patients with *chronic* pulmonary

arterial hypertension: bosentan and the selective ERAs ambrisentan (Gilead

Sciences, Foster City, California) and sitaxsentan (Pfizer, New York, New York). All

three are administered orally. In a meta-analysis of five trials of either bosentan or

sitaxsentan (four of which evaluated bosentan), treatment with ERA over a 12-16

week period improved exercise capacity and dypsnea, and some hemodynamics

measures versus control (placebo in four trials and sildenafil in the fifth) in patients

with mainly idiopathic PAH [237]. In a double-blind randomized controlled trial

called BREATHE-1, patients with idiopathic or connective-tissue disorder-

associated PAH were assigned to two different doses of bosentan or placebo. After a

minimum of twelve weeks of treatment, patients receiving bosentan had

significantly improved six-minute walking distances, improved scores on the Borg

dyspnea index, and longer times to clinical worsening. The ARIES-1 and ARIES-2

trials evaluated three different doses of ambrisentan (10 mg and 5 mg in ARIES-1

and 5 mg and 2.5 mg in ARIES-2) versus placebo in patients suffering from WHO

group I-type PAH [238]. Over the course of the two trials, patients treated with

ambrisentan showed improvements in time to clinical worsening (ARIES-2), World

Health Organization functional class (ARIES-1), Short Form-36 Health Survey score

(ARIES-2), Borg dyspnea score (both studies), and B-type natriuretic peptide (both

studies). The most important side effect of ERAs is hepatic toxicity. Monitoring of

serum hepatic aminotransferase levels is recommended with bosentan, while

sitaxsentan was actually withdrawn from use after several cases of fatal hepatotoxicity. Conversely, ambrisentan has no described hepatotoxicity and monitoring of aminotransferases is not recommended.

Despite its use for over a decade in patients with chronic PAH, little data exists on usage of bosentan in the context of acute CPB-induced (or CPB-worsened) pulmonary hypertension. In a porcine model, administration of intravenous bosentan before CPB was shown to abolish the significant increases in PAP and PVR seen in pigs receiving only saline, with only a moderate decrease in SVR (-19 ± 14.6%, $p<0.05$) [239]. The only study in humans of ERA during or after CPB was a trial in children who had undergone corrective surgery for congenital heart disease [240]. Pulmonary vasodilator therapy was progressively escalated, starting with an increase in FiO_2 to 0.65, then administration of the selective ERA BQ123, and finally addition of inhNO at 20 ppm. PAP and PVR decreased after BQ123 infusion but there was no change in the mean PAP/MAP ratio as MAP was also decreased. Another study evaluating bosentan in patients with chronic PAH also demonstrated systemic vasodilatation during intravenous administration of the ERA [241]. The systemic vasodilatatory effect of ERAs given intravenously and the impracticality of oral administration in a perioperative setting may have limited the study and use of ERAs in the context of cardiac surgery. Inhalation of ERAs has thus far only been described in non-CPB *in vitro* and animal models [242, 243].

Phosphodiesterase Inhibitors

Over the last 15 years, phosphodiesterase (PDE) inhibitors have become well-established weapons in the armamentarium against both acute and chronic

pulmonary hypertension. The phosphodiesterases are intracellular enzymes that are responsible for the inactivation of the NO second messengers cAMP and cGMP to their non-cyclic forms and consequently negating NO's vasodilatory action in the endothelium-VSMC interaction. To date, eleven gene-related families of isozymes (PDE1 to PDE11) have been identified, giving rise to a multitude of isozymes [244]. While most if not all PDE isozymes have cardiovascular effects, the forms that have thus far been identified as having important roles in cardiovascular physiology are PDE3, which hydrolyzes mainly cAMP, and PDE5, which principally hydrolyzes cGMP, with a lesser role for PDE4 [244]. Non-selective inhibition of PDE occurs with a number of methylated xanthines and derivatives which include caffeine and the bronchodilators aminophylline and theophylline. The non-selective PDE inhibitors are also secondarily adenosine receptor antagonists, which may contribute to a number of their effects [245].

Phosphodiesterase-3 Inhibitors

Selective inhibition of individual isozyme forms was first explored in 1977 and has since been proved through the development of a number of selective PDE inhibitors [246]. One of the first selective PDE inhibitors available was amrinone, a PDE3 inhibitor that could be administered orally or intravenously that was a positive inotrope and vasodilator [247]. Amrinone use frequently produced important side effects, including thrombocytopenia and fever, and was subsequently replaced by the more potent PDE3 inhibitor, milrinone, that does not have these negative effects [248]. Since its introduction almost 30 years ago, milrinone has become a well-known medication in the cardiac operating theatre. Milrinone has compared

favorably to placebo in weaning high-risk patients from CPB and was comparable to dobutamine, a catecholamine inotropic agent, for the treatment of low cardiac output after CPB [249, 250]. Conversely, in patients hospitalized for decompensated chronic heart failure treated with intravenous milrinone and outpatients receiving oral therapy, milrinone did not show conclusive benefits and long-term oral milrinone was actually associated with increased mortality, possibly through proarrhythmic effects [251, 252]. Side effects frequently cited in the two randomized, double-blind, placebo-controlled trials included hypotension, syncope, and atrial arrhythmias.

The pulmonary vasodilator effects of milrinone have been described for a number of years. Mean PAP, PVR, and right atrial pressure (RAP) all significantly decreased while indexed cardiac output significantly increased in patients who were treated for low cardiac output after coronary artery bypass grafting (CABG) and/or valve surgery with a bolus and continuous infusion of intravenous milrinone [253]. However it was associated with significant decreases in SVR and MAP but with no mention of hypotension. A recent review of 77 trials using intravenous milrinone in cardiac surgery found frequent reports of hypotension and systemic vasodilatation [254]. With systemic vasodilatation seeming to be a recurring problem with intravenous administration (as with all of the other medications described thus far), researchers began studying the effects of inhaled milrinone. Experimentally in a swine model, inhaled milrinone administered before and during CPB was compared to bolus administration of intravenous milrinone prior to bypass [255]. There were also a placebo group undergoing CPB and a control group without CPB. While there

was no difference in PAP between the two milrinone groups, the inhaled milrinone group demonstrated lesser important decrease in MAP and SVR than the intravenous milrinone group with better prevention of the CPB-induced pulmonary endothelial dysfunction observed in vascular reactivity studies.

Milrinone in Clinical Cardiac Surgery

Unfortunately to this date the number of human studies using inhaled milrinone in cardiac surgery remains relatively small. In 20 patients with preoperative pulmonary hypertension who had undergone CABG and/or valve surgery or heart transplantation, inhaled milrinone and/or inhaled prostacyclin were administered in the intensive care unit [256] in patients who maintained elevated PAP and PVR [257]. Patients receiving nebulization of the milrinone preparation manufactured for intravenous administration for a ten minute period had statistically significant reductions in mean PAP (-6%), PVR (-20%), transpulmonary gradient (TPG) (-15%), and PVR/SVR ratio (-17%). A second group of patients received nebulized prostacyclin for ten minutes and had similar improvements in hemodynamic values (MPAP -6%, PVR -20%, TPG -21%, and PVR/SVR ratio -21%). Addition of inhaled milrinone to ongoing prostacyclin inhalation furthered reduced by 8% as compared to prostacyclin alone. In a larger retrospective single-centre study, 70 patients deemed to be high-risk and who were undergoing a variety of open-heart procedures received inhaled milrinone before (BE group) or after CPB (AF group) with the expectation that it may ease weaning from CPB [258]. Mean PAP before CPB were 25 mmHg and 30 mmHg, respectively, and decreased non-significantly (p=0.10) in the BE group while it increased by 11% in the AF group (p=0.03).

Deterioration in the MAP/mean PAP ratio was also significantly more frequent in the AF group. There was no difference in the change in indexed cardiac output between the two groups.

Phosphodiesterase-5 Inhibitors

More recently, inhibitors of PDE5 were developed. This type of phosphodiesterase is the principal enzyme degrading cGMP within the VSMC. PDE5 inhibitors thus prolong the duration of effect of NO, be it endogenous or not. Interestingly, PDE5 was found to be abundantly expressed in lung tissue [259]. The three different PDE inhibitors of this type, sildenafil (Pfizer Inc., New York, New York), tadalafil (Eli Lilly and Company, Indianapolis, Indiana), and vardenafil (Bayer Pharmaceuticals, Leverkusen, Germany), were all initially approved for the treatment of erectile dysfunction. It was subsequently found in animal models that oral sildenafil could decrease PAP in experimentally-induced acute pulmonary hypertension in lambs and almost abolish the increase in PAP in hypoxia-induced pulmonary hypertension in healthy human volunteers [260, 261]. Administration of oral sildenafil has also been shown to prevent the rebound pulmonary hypertension that is associated with withdrawal of inhNO [262]. The other two PDE5 inhibitors also subsequently demonstrated beneficial effects in pulmonary hypertension.

There is some experience with sildenafil in post-cardiac surgery pulmonary hypertension, although not with tadalafil or vardenafil. In one small study of eight adults, oral sildenafil was administered to treat refractory pulmonary hypertension after mitral valve surgery or placement of left ventricular assist device [263]. All patients were already being treated with optimal conventional pulmonary

vasodilators, including milrinone, inhNO, or nitroglycerin. Mean PAP in this patients

decreased by 9 mmHg at 30 and 60 minutes ($p < 0.05$) without a statistically

significant change in MAP. The other pulmonary vasodilators were typically weaned

within 24 hours of the first dose of sildenafil. Similar benefits were shown in heart

transplant recipients who suffered from preoperative reversible pulmonary

hypertension [264]. The ten patients in the study suffered from acute postoperative

right ventricular dysfunction and received pulmonary vasodilator therapy in order

to improve pulmonary hypertension, reducing RV afterload. All patients were

treated with inhNO until extubation as well as inotropes and intravenous

pulmonary vasodilators. Oral sildenafil therapy permitted weaning of other

pulmonary vasodilators in all patients and there was a trend towards earlier

weaning when sildenafil was administered sooner. Thus, oral sildenafil therapy has

demonstrated benefits when used in the ICU for postoperative pulmonary

hypertension.

Unfortunately, oral therapy is relatively impractical in the operating room.

Intravenous use was therefore studied in the hope that the relative specificity of

PDE5 to the pulmonary vasculature would limit the systemic hypotension that can

be problematic with intravenous use of the other pulmonary vasodilators. Two

small trials in children undergoing surgery for congenital heart defects used

intravenous sildenafil. The first trial treated infants with increased PVR who had

undergone corrective surgery for lesions with left-to-right shunts [265]. Within two

hours of arrival in the ICU, the children were serially treated with inhNO at 20 ppm,

step-wise increasing doses of intravenous sildenafil, followed by a second treatment

with inhNO. Intravenous sildenafil tended to lower indexed PVR more than inhNO

alone (25.8% vs. 14.6%, *p*<0.09). However, after infusion of the largest dose of

sildenafil (0.25 mg/kg), there was increase in intrapulmonary shunting and a

statistically, but not clinically, significant drop in systemic pressure (a fall of 7.3 ±

2.5 mmHg, *p*<0.01). The other trial was a double blind, multi-center, placebo-

controlled, dose-ranging, parallel group study of three different doses of

intravenous sildenafil [266]. Patient accrual was slow and the study was

unfortunately terminated after only five patients in the placebo group and four

patients in each sildenafil dosage group were recruited. Patients receiving sildenafil

were analyzed together. Mean PAP decreased significantly in the sildenafil group

(46 ± 11 to 35 ± 6 mmHg vs. 49 ± 12 to 49 ± 17, *p*=0.027). There was no difference

in systemic pressures. Other clinically significant benefits seen in the sildenafil

group were shorter median time to extubation (3 versus 8 days, p = 0.023) and

intensive care unit stay (6 versus 15 days, p = 0.008).

Anti-inflammatory Agents and Transduction Pathway Inhibitors

Many of the treatments described above have shown effectiveness in treating or

preventing the development of pulmonary hypertension post-CPB. However, having

considered the mechanisms underlying the development of post-CPB pulmonary

hypertension, one may come to realize that all of these therapies serve only as

symptomatic treatment simply acting as vasodilators without targeting the reaction

to CPB that is at the root of pulmonary hypertension.

Corticosteroids

Following the reasoning that the reaction to CPB is in great part inflammatory, corticosteroids have been employed to reduce this response. As agents that have been long used in a number of domains of medicine, including over 40 years in cardiac surgery [267], their anti-inflammatory properties are well-known, are generally well-tolerated by patients when used for short courses, and easily available at low cost. In studies of patients undergoing cardiac surgery, they have been shown to attenuate expression of mediators of inflammation, lead to less leukocyte activation and less tissue plasminogen activator activity, and result in less vasodilatation and improved markers of peripheral perfusion [268-270]. In a small study, preoperative and intraoperative corticosteroids or only intraoperative corticosteroids were administered to piglets undergoing CPB with prolonged hypothermic circulatory arrest followed by rewarming and were compared to controls treated with saline [10]. The animals treated with both preoperative and intraoperative corticosteroids had no increase in PVR, lower plasma levels of ET-1, and higher levels of IκBα than controls. Notably, an extensive review of the use of corticosteroids in patients undergoing cardiac surgery with CPB has cast doubt however on the benefits of these medications and revealed possible adverse effects [271].

Calpain Inhibitors

Given the evidence of benefit but also possible harm with the use of corticosteroids, researchers attempted to determine the mechanisms underlying the benefit derived with this treatment in order to produce a more effective therapy. The protein calpain appears to play a role in ischemia-reperfusion injury in a number of organ

systems [272]. Calpain may also contribute to the lung ischemia-reperfusion injury and pulmonary endothelial dysfunction associated with CPB and administration of an exogenous inhibitor was shown to reverse the increase in PVR and to blunt the increase in ET-1 levels seen after CPB with prolonged hypothermic circulatory arrest in piglets [13]. While a direct link between calpain and NF-κB activity was only proposed in this article, calpain inhibitor I was shown in another study to attenuate the binding of activated NF-κB to DNA and the degradation of IκBα, IκBβ, and IκBε [273]. NF-κB's role as a transcription factor for ET-1 was discussed earlier.

Nuclear Factor κB Inhibitors

As a result of this evidence of the role of NF-κB in the development of pulmonary hypertension, some authors have begun studying specific inhibitors of NF-κB. The NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) was tested in a monocrotaline-induced pulmonary hypertension model in rats [11]. Two groups received the toxin monocrotaline and either saline or PDTC. A third group received only saline. PDTC greatly attenuated the increase in mean PAP seen with monocrotaline treatment. Treatment with PDTC also reduced right ventricular hypertrophy, pulmonary vascular remodeling, and restored the decrease in protein levels of IκBα in the lung tissues seen with monocrotaline treatment. A different NF-κB inhibitor, SN50, was subsequently tried in a porcine CPB model [12]. This peptide inhibits NF-κB translocation and activation. Authors noted that the dose was chosen for partial rather than complete blockade of NF-κB activity in order to reduce detrimental NF-κB-activated responses to ischemia and reperfusion without stimulating cell injury that may be associated with complete blockade. CPB was instored in these animals

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and they were cooled to 18°C and then underwent circulatory arrest for 120

minutes before rewarming over a period of 45 minutes and subsequent weaning of

CPB. The animals were then kept under general anesthesia for another 120 minutes.

At the end of this period, PVR (dynes•sec^{-1}•cm^{-5}) had increased to 369 ± 104 from

124 ± 59 at baseline in the untreated piglets (p=0.001) compared with SN50-treated

animals (100 ± 24 at baseline and 169 ± 88 at 120 minutes, p=0.1). NF-κB activity

was reduced by 74% in the left ventricle tissue of SN50-treated compared with

SN50-untreated animals (p<0.001). Plasma endothelin-1 (pg/mL), increased from

2.1 ± 0.4 to 14.2 ± 5.7 in untreated animals (p=0.004) but the increase was

significantly smaller, to only 4.5 ± 2, with SN50 treatment (p=0.005). SN50 also

abolished the fall in MAP at 120 minutes post-CPB seen in controls and SN50-

treated animals had preserved post-CPB systolic and diastolic function measured by

maximum and minimum dP/dt, tau, and preload recruitable stroke work.

Furthermore, this reduction in cardiac dysfunction was associated with improved

oxygen delivery. Thus, selective inhibition of NF-κB with SN50 may alleviate

pulmonary hypertension and maintain oxygen delivery by more specifically

targeting the mechanisms underlying ischemia-reperfusion injury.

HYPOTHESIS & OBJECTIVES

A porcine model of post-CPB pulmonary endothelial dysfunction and pulmonary hypertension has been developed in our laboratory. It has been used in multiple studies since 2004 in order to independently examine the effects of different agents including intravenous and inhaled milrinone, intravenous and inhaled sildenafil, and inhaled prostacyclin administered before, during, or after CPB [231, 255, 274]. Benefit from use of these agents was demonstrated in all three studies; however no study compared different agents, only different modalities and timings of administration. Moreover, systemic hypotension was a frequent adverse effect stemming from the vasodilator properties intrinsic to all of the medications.

Inhibitors of NF-kB have demonstrated some promise in the prevention of pulmonary hypertension in some animal models, including a porcine CPB model [12]. Inhibition of NF-kB translocation and activation with the agent SN50 in this model abolished the increase in PVR and the fall in MAP post-CPB while diminishing endothelin-1 production and reducing cardiac dysfunction.

The hypothesis underlying this work is that SN50 better alleviates post-CPB pulmonary hypertension and provides better cardiac output and oxygenation in a porcine CPB model compared to inhaled milrinone, inhaled sildenafil, and placebo by improving or reversing the pulmonary endothelial dysfunction that occurs post-CPB.

MATERIALS & METHODS

All experiments were performed using crossbred Landrace-Yorkshire swine (Ferme Marc Lavallée, Montreal, QC, Canada) of either gender, aged 8 weeks and weighing 40 kg. Animals were divided into four groups, receiving one of the four treatments described below, specifically inhaled milrinone, inhaled sildenafil, inhaled saline solution placebo, and intravenous SN50. Six animals were included in each group. Animals were maintained and tested in accordance with the recommendations of the Guidelines on the Care and Use of Laboratory Animals issued by the Canadian Council on Animals. The piglets were fasted for 12 hours prior to surgery and sedated with intramuscular ketamine hydrochloride (25 mg/kg; Ayerst Veterinary Laboratories, Guelph, ON, Canada) and xylazine (10 mg/kg; Boehringer Ingelheim, Burlington, ON, Canada) and induction was achieved using mask ventilation with 2% isoflurane (Abbott Laboratories Limited, St-Laurent, QC, Canada). They were subsequently intubated and mechanically ventilated with a constant oxygen and air mixture (3:2, or FiO2 = 0.66) at 14 breath strokes/min and tidal volume of 6–8 mL/kg. Anesthesia was maintained with 1% isoflurane inhalation. Arterial and venous blood gases were measured at regular intervals and maintained within physiological limits by adjusting the ventilation rate and tidal volume. An electrocardiogram was recorded from four subcutaneous limb and one precordial electrodes. The jugular vein and femoral artery were cannulated to obtain central venous access and to monitor arterial pressure, respectively. A Swan-Ganz pulmonary artery catheter (Edwards Lifesciences, Irving, CA) was inserted through the jugular vein to measure wedge, central venous, and pulmonary artery pressures and cardiac output.

A median sternotomy was performed and the pericardium was opened, exposing the heart. The experimental medication was administered as described below. After heparin administration (400 IU/kg), a double purse string was made on the proximal ascending aorta, and a single purse string was made on the right atrium. A blood sample was then drawn from the right atrium, and anticoagulation was assessed by measuring activated coagulation time (ACT) by using the Hemochron 801 (Technidyne, Dorval, Quebec, Canada). The aorta and right atrium were cannulated when the ACT was greater than 300 seconds with 22F and 29/29F double-staged cannulas (DLP, Inc, Grand Rapids, Mich), respectively. After cannulation, CPB was initiated when the ACT was greater than 400 seconds. Ventilation was discontinued throughout the CPB period. Anesthesia was maintained intravenously with a continuous infusion of propofol (0.1-0.2 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}; Pharmascience, Inc, Montreal, Quebec, Canada). The CPB circuit consisted of a hollow-fiber membrane oxygenator with an incorporated filtered hardshell venous reservoir (Monolyth; Sorin, Irvine, Calif), a heater-cooler, and a roller pump (Sarns 7000, Ann Harbor, Mich). The circuit was primed with 500 mL of Pentaspan (10% Pentastarch; DuPont Pharma, Inc, Mississauga, Ontario, Canada), 250 mL of Ringer's lactate solution, 5000 IU of heparin, 12.5 g of mannitol, and 15/mEq of sodium bicarbonate. After initial stabilization, the pump flow was adjusted to maintain an index of 2.4 L $\cdot$ min^{-1} $\cdot$ m^{-2} and assessed on the basis of venous gases to maintain a mixed venous saturation of greater than 60%. Mean systemic arterial pressure was maintained between 50 and 70 mm Hg with Ringer's lactate and discrete boluses of 50 to 200 μg of neosynephrine (Cayman Chemical Co, Ann Arbor, Mich). The

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temperature was allowed to drift to 36°C. The heart was left beating and empty. No aortic crossclamping or cardioplegia was used. Before CPB weaning, swine were rewarmed to 38°C (normal porcine body temperature). After 90 minutes of CPB, mechanical ventilation and isoflurane-induced anesthesia were reinstituted, and the animals were weaned from CPB. Normal circulation was restored for 60 minutes, at which time the animal was exsanguinated into the cardiotomy reservoir. The heart and lungs were excised en bloc and immediately immersed in a cold modified Krebs-bicarbonate solution (NaCl, 118.3 mmol/L; KCl, 4.7 mmol/L; $MgSO_4$, 1.2 mmol/L; KH_2PO_4, 1.2 mmol/L; glucose, 11.1 mmol/L; $CaCl_2$, 2.5 mmol/L; $NaHCO_3$, 25 mmol/L; and EDTA: ethylenediamine tetraacetic acid, 0.026 mmol/L).

Treatment Medications

After median sternotomy but before administration of heparin, animals received the treatment medication. Nebulized medications (NaCl, milrinone, and sildenafil) were administered using a conventional in-line nebulizer (Salter Labs, Arvin, California) connected to the inspiratory limb of the ventilator circuit. Milrinone (Primacor; Sanofi-Synthelabo Canada, Inc, Markham, Ontario, Canada) was given as a dilution of 2 mg of milrinone, 1 mg/mL, diluted in 8 mL of normal saline (200 µg/mL). A bolus of 1.8 mg (50-90 µg/kg) was administered over a period of 15 minutes before heparinisation and cannulation. Similarly, a 10 mg bolus of sildenafil (0.5 mg/kg; Viagra, Pfizer, Sandwich, UK) diluted in 20 mL of buffer (0.038 mol/L sodium acetate) was nebulized over 15 minutes and administered through the inspiratory limb of the ventilator circuit for animals in the second group. Animals in the placebo group received 10 cc of nebulized 0.9% NaCl solution using the same technique.

Animals in the final group received a 100 μg/kg bolus of the NF-κB inhibitor SN-50 (Enzo Life Sciences, Farmingdale, New York) diluted in water to a concentration of 200 μg/mL. This bolus was administered intravenously before heparinisation and cannulation.

Monitoring

As outlined above, all animals underwent invasive monitoring using a femoral artery catheter and central venous cannulation of the internal jugular vein using a Swan-Ganz pulmonary artery catheter, permitting continuous monitor of systemic blood pressure, pulmonary artery pressure, and central venous pressure. Continuous five-lead ECG monitoring was also used in all cases. Hemodynamic measures were noted and cardiac output and pulmonary capillary wedge pressure were measured at baseline; after medication administration; at 15, 45, and 75 minutes of CPB; and at 30 minutes and 60 minutes of reperfusion. Cardiac output was measured with the pulmonary artery catheter using the thermodilution method. At these same instances, arterial and venous blood gases were drawn for analysis.

Vascular Reactivity Studies

Less than 10 min after en bloc excision, the heart was removed and the main pulmonary artery was dissected. Second-degree branches of the pulmonary arteries were isolated and dissected free of connective and adventitial tissue and divided into rings (4-mm wide; 16 rings per animal). All rings were placed in organ chambers (Emka Technologies Inc., Paris, France) filled with 20 mL of modified

Krebs-bicarbonate solution continuously heated at 37°C and oxygenated with a carbogen mixture (95% O_2 and 5% CO_2). The rings were suspended between two metal stirrups with the upper one connected to an isometric force transducer, itself connected to a signal amplifier and then allowed to stabilize for 30 minutes. Data were collected with biological signal data acquisition software (IOX 1.700; Emka technologies Inc., Paris, France). Each arterial ring was stretched to the optimal point of its active length-tension curve (4.0 g) as determined by measuring the contraction to potassium chloride (KCl; 30 mmol/L) at different levels of stretch (data not shown). The maximal contraction of rings was then obtained with addition of potassium chloride (KCl 60 mmol/L). After stabilization, all baths were washed twice with modified Krebs-bicarbonate solution and indomethacin (10^{-5} mmol/L; to prevent production of endogenous prostanoids) was added in each bath. After 45 min of stabilization, phenylephrine (PE, range 2×10^{-7} mol/L to 3×10^{-6} mol/L) was added to obtain a contraction averaging 50% of the maximal contraction to KCl.

Endothelium-dependent Relaxations

The NO-mediated relaxation pathway was studied by constructing concentration-response curves to acetylcholine (10^{-9} to 10^{-5} mol/L; an agonist of M_2 receptors coupled to G_i proteins that leads to NO release) and to bradykinin (10^{-12} to 10^{-6} mol/L; an agonist of B_2 receptors coupled to G_q proteins that cause release of NO and EDHF).

Endothelium-independent Relaxations

At the end of the experiment, endothelium-independent relaxations were studied with the use of a bolus of 10^{-5} mol/L sodium nitroprusside (SNP), an NO donor.

RESULTS

		Baseline	SEM	Post-Medication	SEM	CPB 15 min	SEM
BPs	SN50	100.0	15.7	74.3	8.5	78.8	6.3
(mmHg)	Milrinone	81.5	7.2	78.0	9.2	77.7	16.3
	Placebo	86.7	8.2	73.5	10.5	68.5	6.5
	Sildenafil	103.3	12.0	85.7	9.8	73.3	9.7
MAP	SN50	80.8	11.7	61.8	7.5	73.8	6.4
(mmHg)	Milrinone	61.7	4.9	60.5	8.8	76.8	10.5
	Placebo	65.5	4.0	55.5	5.5	67.9	6.1
	Sildenafil	74.3	9.8	58.0	9.0	64.8	7.5
HR	SN50	91.7	4.1	112.3	4.7	117.0	14.9
(beats/min)	Milrinone	92.6	4.6	97.0	14.1	79.3	12.3
	Placebo	101.5	7.9	106.5	16.5	126.0	37.1
	Sildenafil	94.3	9.4	# 80.8	4.5	126.7	28.4
PAPs	SN50	16.3	1.1	17.2	1.6		
(mmHg)	Milrinone	16.8	2.4	14.3	2.3		
	Placebo	18.7	2.3	20.5	7.5		
	Sildenafil	19.5	5.2	18.3	0.9		
PAPm	SN50	14.7	1.2	14.5	1.6		
(mmHg)	Milrinone	11.4	1.3	10.8	2.5		
	Placebo	15.3	1.2	17.5	5.5		
	Sildenafil	14.0	4.4	12.8	2.1		
mPAP/MAP	SN50	0.20	0.02	0.24	0.02		
	Milrinone	0.20	0.02	0.18	0.03		
	Placebo	0.23	0.02	0.31	0.04		
	Sildenafil	0.21	0.04	0.25	0.02		
Wedge	SN50	6.7	1.9	$ 6.8	1.3		
(mmHg)	Milrinone	4.8	1.2	3.8	0.9		
	Placebo	4.0	0.5	4.0	2.0		
	Sildenafil	5.7	1.8	$ 7.6	0.9		
CVP	SN50	4.2	1.4	3.2	1.2		
(mmHg)	Milrinone	2.3	0.7	3.0	1.2		
	Placebo	3.8	0.9	3.5	2.5		
	Sildenafil	2.8	0.6	6.2	2.6		
CO	SN50	3.1	0.2	3.0	0.2	3.1	0.1
(L/min)	Milrinone	2.6	0.3	3.0	0.2	3.5	0.3
	Placebo	3.6	0.9	4.2	1.6	3.4	0.3
	Sildenafil	3.4	0.3	2.8	0.4	3.1	0.5

A-a	SN50	210.2	16.0	277.3	52.1		
(mmHg)	Milrinone	226.3	23.7	251.8	16.9		
	Placebo	178.5	32.6	212.8	35.8		
	Sildenafil	255.1	12.1	317.7	35.5		
Lactate	SN50	1.8	0.2	1.6	0.3	3.1	0.5
(mmol/L)	Milrinone	2.2	0.2	2.3	0.5	3.9	0.3
	Placebo	1.9	0.6	1.7	0.3	3.3	1.1
	Sildenafil	2.7	0.4	3.9	0.8	5.0	0.9
Venous Sat	SN50	78.4	3.3	76.5	2.3	72.8	5.4
(%)	Milrinone	75.3	3.5	58.5	19.2	70.8	6.6
	Placebo	72.5	11.5	76.7	3.3	74.7	5.7
	Sildenafil	84.3	2.3	73.0	9.3	74.8	4.9

		CPB 45 min		CPB 75 min		30 min post CPB	
			SEM		SEM		SEM
BPs	SN50	85.5	8.2	73.6	6.4	90.4	5.6
(mmHg)	Milrinone	69.7	11.8	65.0	4.0	70.8	10.7
	Placebo	61.5	2.5	78.5	4.5	72.0	12.0
	Sildenafil	76.3	7.4	69.8	7.2	66.0	10.2
MAP	SN50	75.2	7.5	68.0	6.1	64.8	4.3
(mmHg)	Milrinone	67.7	8.2	67.0	5.2	49.3	6.2
	Placebo	60.4	3.5	73.6	3.4	56.8	8.1
	Sildenafil	69.2	6.6	66.5	6.1	50.0	6.7
HR	SN50	105.7	5.5	117.8	11.2	85.2	20.0
(beats/min)	Milrinone	90.5	10.7	89.8	11.4	103.5	10.8
	Placebo	135.5	33.6	105.8	11.8	109.0	2.0
	Sildenafil	106.5	11.9	108.0	15.4	111.5	11.8
PAPs	SN50					18.4	1.6
(mmHg)	Milrinone					17.8	4.4
	Placebo					21.5	1.5
	Sildenafil					20.8	1.9
PAPm	SN50					15.8	1.4
(mmHg)	Milrinone					14.2	3.7
	Placebo					15.8	1.5
	Sildenafil					17.3	3.2
mPAP/MAP	SN50					0.25	0.03
	Milrinone					0.37	0.18
	Placebo					0.30	0.05
	Sildenafil					0.43	0.09
Wedge	SN50					4.0	0.8

(mmHg)	Milrinone					4.3	0.7
	Placebo					3.5	0.5
	Sildenafil					# 8.0	1.6
CVP	SN50					5.0	1.5
(mmHg)	Milrinone					3.3	0.9
	Placebo					4.8	1.4
	Sildenafil					3.2	1.2
CO	SN50	3.2	0.1	3.0	0.2	2.7	0.5
(L/min)	Milrinone	3.4	0.3	3.3	0.3	3.9	0.6
	Placebo	3.2	0.4	3.2	0.4	3.1	0.1
	Sildenafil	3.2	0.2	3.2	0.2	3.9	0.7
A-a	SN50					207.1	8.0
(mmHg)	Milrinone					201.1	24.9
	Placebo					255.5	40.2
	Sildenafil					#294.9	44.8
Lactate	SN50	2.9	0.5	3.1	0.4	3.5	0.5
(mmol/L)	Milrinone	3.8	0.6	3.8	0.7	3.9	0.8
	Placebo	3.9	1.9	4.8	2.0	4.6	1.4
	Sildenafil	4.3	0.7	4.4	0.9	5.8	1.3
Venous Sat	SN50	72.2	3.7	72.7	2.7	65.8	6.7
(%)	Milrinone	77.0	3.6	70.3	4.2	67.5	3.2
	Placebo	73.0	4.0	78.3	4.4	68.3	5.9
	Sildenafil	76.5	1.5	73.3	1.7	67.5	6.6

60 min post CPB

			SEM	
BPs	SN50	80.5	11.3	*
(mmHg)	Milrinone	63.8	9.0	
	Placebo	65.7	2.0	*
	Sildenafil	53.0	9.5	*
MAP	SN50	56.0	8.4	
(mmHg)	Milrinone	50.0	6.6	
	Placebo	47.5	1.4	
	Sildenafil	41.3	6.0	*
HR	SN50	109.2	7.1	
(beats/min)	Milrinone	104.8	11.9	
	Placebo	123.3	22.7	
	Sildenafil	120.5	16.4	*
PAPs	SN50	20.4	2.0	
(mmHg)	Milrinone	18.5	5.9	

	Placebo	21.0	2.9	
	Sildenafil	18.0	1.2	
mPAP	SN50	16.4	1.6	
(mmHg)	Milrinone	14.8	4.3	
	Placebo	17.0	2.4	
	Sildenafil	16.0	2.1	*
mPAP/MAP	SN50	0.34	0.06	
	Milrinone	0.43	0.23	*
	Placebo	0.37	0.05	
	Sildenafil	0.45	0.08	*
Wedge	SN50	4.0	1.0	*
(mmHg)	Milrinone	3.7	1.4	
	Placebo	4.8	1.2	
	Sildenafil	#7.5	0.3	
CVP	SN50	4.6	1.3	
(mmHg)	Milrinone	2.5	1.4	
	Placebo	4.0	0.5	
	Sildenafil	3.5	1.1	
CO	SN50	2.4	0.4	
(L/min)	Milrinone	3.7	0.4	
	Placebo	2.9	0.3	
	Sildenafil	3.2	0.6	
A-a	SN50	219.8	21.3	
(mmHg)	Milrinone	222.4	44.8	
	Placebo	207.9	32.5	
	Sildenafil	261.7	49.9	
Lactate	SN50	3.1	0.5	*
(mmol/L)	Milrinone	3.8	1.1	*
	Placebo	3.9	0.9	*
	Sildenafil	5.5	1.6	*
Venous Sat	SN50	55.4	5.6	
(%)	Milrinone	71.5	8.0	
	Placebo	66.7	2.7	
	Sildenafil	64.5	3.7	

Table 1. Hemodynamic data throughout the experiment.
CPB, mean cardiopulmonary bypass; *BPs*, systolic arterial pressure; *MAP*, mean arterial pressure; *HR*, heart rate; *mPAP*, pulmonary artery pressure; *mPAP/MAP*, ratio of mean pulmonary to mean systemic arterial pressures; *CVP*, central venous pressure; *CO*, cardiac output; *A-a*, alveolar-arterial oxygen gradient; *Venous Sat*, mixed venous blood oxygen saturation; *, $p < 0.05$ vs. baseline; #, $p < 0.05$ vs. other groups at same time point; $, $p < 0.05$ vs. Milrinone and Placebo.
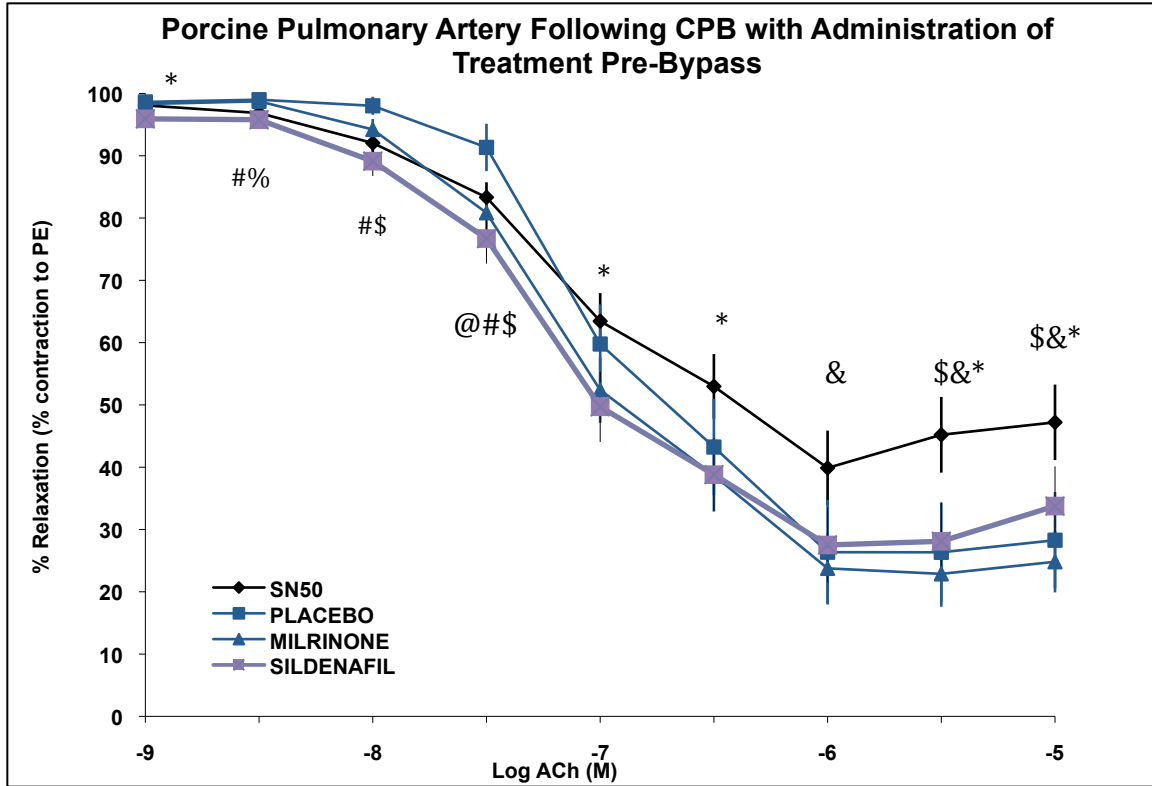
95

Figure 7. Cumulative dose-reponse curve in response to acetylcholine of rings of porcine pulmonary artery. Data expressed as means ± SEM. @ $p<0.05$ Placebo vs Milrinone, # $p<0.05$ Placebo vs Sildenafil, $ $p<0.05$ Placebo vs SN50, % $p<0.05$ Milrinone vs Sildenafil, & $p<0.05$ Milrinone vs SN50, * $p<0.05$ Sildenafil vs SN50
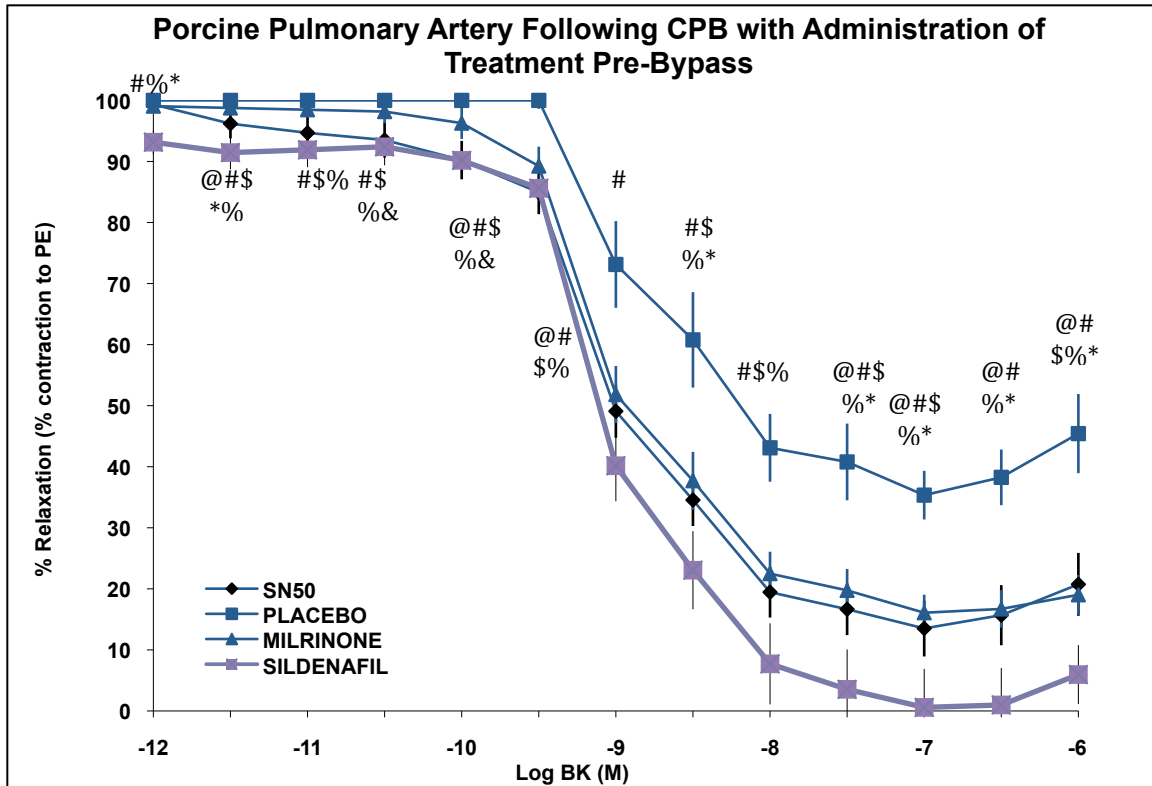
Figure 8. Cumulative dose-reponse curve in response to bradykinin of rings of porcine pulmonary artery. Data expressed as means ± SEM. @ $p<0.05$ Placebo vs Milrinone, # $p<0.05$ Placebo vs Sildenafil, $ $p<0.05$ Placebo vs SN50, % $p<0.05$ Milrinone vs Sildenafil, & $p<0.05$ Milrinone vs SN50, * $p<0.05$ Sildenafil vs SN50

Hemodynamic and Biochemical Results

Systemic Hemodynamic Parameters, Cardiac Output, and Heart Rate

There was a statistically significant decrease in systolic blood pressure (BPs) from baseline to 60 minutes after CPB in the SN50, placebo, and sildenafil groups (100.0±15.7 vs. 80.5±11.3 mmHg, 86.7±8.2 vs. 65.7±2.0 mmHg, and 103.3±12.0 vs. 53.0±9.5 mmHg, respectively). However, this fall in pressure was only associated with a statistically significant fall in MAP over the same period in the sildenafil group (74.3±9.8 vs. 41.3±6.0 mmHg). Simultaneously, heart rate increased significantly from 94.3±9.4 beats per minute at baseline to 120.3±16.4 beats per

minute at 60 minutes of reperfusion, with no significant change in heart rate for the other groups. Heart rate was statistically significantly lower in the sildenafil group immediately after administration of the medication compared to the other groups (80.8±4.5 vs. 112.3±4.7, 97.0±14.1, and 106.5±16.5 beats per minute for SN50, milrinone, and placebo groups, respectively). There was no difference between groups or over time in cardiac output or central venous pressure.

Pulmonary Hemodynamic Parameters

There was a statistically significant increase in mean PAP in the sildenafil group from 14.0±4.4 mmHg at baseline to 16.0±2.1 mmHg 60 minutes after CPB. The change in mean PAP over the course of the experiment was not statistically significant in the other groups, nor was the difference between groups at different times. There was no difference between or within groups in systolic PAP. The mean PAP/MAP ratio increased significantly from baseline to 60 minutes of reperfusion in the sildenafil and milrinone groups (0.21±0.04 vs. 0.45±0.08 and 0.20±0.02 vs. 0.43±0.23, respectively). The pulmonary capillary wedge pressure (PCWP) was statistically significantly higher in the sildenafil and SN50 groups after administration of the treatment medications (7.6±0.9 and 6.8±1.3 mmHg, respectively) compared to the milrinone and placebo groups (3.8±0.9 and 4.0±2.0 mmHg, respectively). At 30 and 60 minutes of reperfusion, the PCWP was statistically higher in the sildenafil group compared to the other groups. However, only the SN50 group demonstrated a significant change in PCWP over the course of the experiment (6.7±1.9 mmHg at baseline vs. 4.0±1.0 mmHg at 60 minutes post-CPB).

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Oxygenation

There was a statistically significant difference in alveolo-arterial (A-a) gradient between the SN50 and milrinone groups and the sildenafil and placebo groups at 30 minutes post-CPB. The SN50 and milrinone groups had A-a gradients of 207.1±8.0 and 201.1±24.9 mmHg, respectively, whereas the sildenafil and placebo groups had A-a gradients of 294.9±44.8 and 255.5±40.2 mmHg, respectively. There was no other statistical difference between or within groups.

Biochemical Data

There was a statistically significant increase in serum lactate levels over the course of the experiment in the SN50, milrinone, and placebo groups, with the peak at 30 minutes after CPB followed by a slight decrease at 60 minutes. The sildenafil group followed the same trend, but did not reach statistical significance (p=0.075). There was no statistical difference between or within groups in mixed venous oxygen saturation.

Vascular Reactivity Studies

Endothelial-Dependent Relaxations

Acetylcholine-induced relaxations were statistically significantly better in the sildenafil group compared to the placebo group at the lower concentrations of acetylcholine but this effect was lost at the higher concentrations. SN-50 produced statistically significantly lesser relaxations than sildenafil, milrinone, and placebo at the higher concentrations of acetylcholine, while the difference was only significant with respect to sildenafil at the mid-range concentrations.

Bradykinin-induced relaxations were significantly increased in the sildenafil,

milrinone, and SN-50 groups compared to placebo. At higher concentrations of

bradykinin, relaxations induced by sildenafil were also significantly greater than

those induced by either SN-50 or milrinone. Relaxations were similar between

milrinone and SN-50.

Endothelium-Independent Relaxations

There was no difference observed between the groups in the relaxations to SNP,

with all pulmonary artery rings achieving 100% relaxation (data not shown).

DISCUSSION

The aim of this study was to compare the effects of SN-50, milrinone, and sildenafil to placebo on the pulmonary hypertension observed after CPB, looking at hemodynamic and oxygenation parameters as well as pulmonary endothelial dysfunction. The main findings were: a) an increase in different measures of PAP post-CPB only in the sildenafil and milrinone group that was not significantly different between the groups, associated with a significant drop in MAP in the sildenafil group; b) elevations of PCWP, principally in the sildenafil group; c) transitory improvement in oxygenation after CPB in the SN-50 and milrinone groups; and d) improved endothelium-dependent relaxations in response to BK with all therapies as compared to placebo, especially with sildenafil.

Hemodynamics

The increase in mean PAP in response to exposure to CPB was non-significant in three of the four groups tested. Only the sildenafil group demonstrated a statistically significant increase in mean PAP over the course of the experiment. However, the absolute increase in mean PAP in mmHg (approximately 2 mmHg) would generally be insufficient to have any impact on hemodynamics or oxygenation. More significant perhaps is the change in mean PAP/MAP ratio. It is frequently used in congenital cardiology and cardiac surgery to stratify the severity of pulmonary hypertension given the intimate relationship of the pulmonary and systemic circulations [275]. Thus, for the same pulmonary artery pressure, lower systemic arterial pressure would indicate more severe pulmonary hypertension. While generally expressed clinically in the form of mean PAP/MAP, the reciprocal of this ratio was studied in order to determine its utility as a hemodynamic parameter in

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adult cardiac surgery and its value for predicting hemodynamic complications [276]. Data from over 1,500 adults undergoing cardiac surgery was studied and a multiple stepwise logistic regression analysis showed lower MAP/mean PAP ratio to be an independent predictor of the composite index of hemodynamic complications (odds ratio 1.3, CI, 1.1-1.5, $p<0.0001$). Additionally, the authors found that this value was not influenced by the induction of general anesthesia, which is known to be effective for decreasing absolute PAP. In this study, the mean PAP/MAP ratio increased from baseline to 60 minutes post-CPB in two groups only: in the sildenafil group passing from 0.21±0.04 to 0.45±0.08 and in the milrinone group, passing from 0.20±0.02 vs. 0.43±0.23. However, there were no differences between the groups at any time point during the experiment. The increase in mean PAP/MAP ratio in the sildenafil group may have partly been driven by the significant decrease in MAP. Notably cardiac output was not significantly different, suggesting systemic vasodilation as the cause of decreased MAP.

While it could be expected to see changes in PAP post-CPB as compared to baseline, this difference has not been seen universally when this porcine model has been used. In one study, one group of animals was administered inhaled prostacyclin before and during CPB and compared to an untreated CPB group and a no CPB control group [231]. The CPB group demonstrated a statistically significant increase in mean PAP at zero and ten minutes after the cessation of CPB, whereas this increase was not seen in the prostacyclin/CPB group. Notably, the hemodynamic parameters were monitored for 60 minutes after weaning of CPB and the difference in PAP was no longer significant from 15 minutes after CPB onwards. In a similar

paper studying the effects of intravenous and inhaled milrinone in the same porcine CPB model, there was no difference seen in PAP within or between the groups over the course of the experiment [255]. In this study, hemodynamic parameters were measured at 30 and 60 minutes post-CPB, as in the current study. A third study examined the effects of inhaled and intravenous sildenafil within this porcine model [274]. After a similar duration of CPB, there was a statistically significant increase in mean PAP in the untreated CPB group, which was not seen in the groups receiving inhaled or intravenous sildenafil prior to CPB. The animals receiving inhaled sildenafil after CPB only had a statistically non-significant increase (p=0.37) in mean PAP at 30 minutes of reperfusion, which increased further at 60 minutes of reperfusion but remained non-significant (p-value not available). Thus, in this model, 90 minutes of normothermic CPB does not consistently document increased PAP at 30 and 60 minutes after CPB and concords with the results seen in this study.

In a slightly different porcine CPB model, swine underwent CPB at 28°C with 30 minutes of aortic crossclamping and cardiac arrest induced with hyperkalemic antegrade cardioplegia [277]. Animals were randomized to receive intravenous milrinone, the sildenafil analog UK343-664, or normal saline placebo 10 minutes after weaning from CPB. All three groups had statistically significantly increased mean PAP 10 minutes after weaning of CPB. Only the sildenafil analog group had a statistically significant decrease in PVR at five and 10 minutes after drug administration. However, at 30 minutes mean PAP and PVR in all groups was not significantly different from baseline. Taken with the study discussed earlier of inhaled prostacyclin, these findings suggest that there may be early, short-lived

post-CPB increases in mean PAP and PVR that are not captured by measures done at 30 minutes or later.

Another consideration is that, unlike in humans where clear definitions of pulmonary hypertension have been established (in terms of absolute and relative pressures), no such definition exists in swine. However, using the mean PAP/MAP ratio, parallels can be drawn with humans. While only the sildenafil and milrinone group had increase mean PAP/MAP ratio at the end of reperfusion as compared to baseline, all groups had mean PAP of at least one third of MAP, a value that most clinicians would agree is consistent with at least mild pulmonary hypertension. This study was the only one using this porcine model to report a mean PAP/MAP ratio permitting this comparison.

The porcine model used in studying transcription factor modulation such as calpain inhibition and NF-κB inhibition more consistently demonstrated increases in PVR than our model [12, 13]. However, there are important differences between these two animal models. Firstly, the duration of "non-physiologic" circulation is much longer in those studies, with 120 minutes of DHCA preceded and followed by approximately 40 minutes each of cooling and rewarming on CPB. This is much longer than the 90 minutes of CPB in our model, which was conducted at normothermia which may better preserve homeostasis and enzyme function. As described earlier, the inflammatory reaction to CPB and the extent of pulmonary ischemia-reperfusion damage is one of several important determinants of post-CPB pulmonary hypertension and is more severe with longer CPB duration. CPB duration has been shown in humans to be an independent risk factor for pulmonary

complications, multi-organ failure, and postoperative death, among others [278]. SN-50 may also be more potent under these circumstances than in a shorter CPB run without DHCA, as the greater inflammatory and ischemic insults and longer procedural time may produce greater changes at the transcription factor level modulated by the NF-κB inhibitor. Additionally, hypothermia causes pulmonary vasoconstriction and may reduce the pulmonary blood flow which is already limited on CPB [279]. Finally, the animals in the Duffy studies were younger than those used in our model. While the age of the piglets was not specified beyond using the term "neonatal," they were significantly younger than the animals used in our model (~8 weeks of age). Given that evidence suggests that porcine lungs exhibit a high degree of maturity at birth with rapid pulmonary development over the first two weeks of life [280], the difference in age may not be physiologically significant.

Interpretation of the results of this study must also be tempered by considering other factors affecting the pulmonary vasculature that are present in clinical cardiac surgery but not in our porcine model. While these factors were eliminated in order to provide a more controlled experimental model, they may be important in clinical cardiac surgery. First, there was no reversal of heparinisation with protamine nor in our model nor in the other porcine model discussed. Heparin reversal with protamine after the conclusion of CPB is universal in cardiac surgery and its accidental omission is usually associated with major intraoperative hemorrhage. As described earlier, the heparin-protamine complex induces release of the pulmonary vasoconstrictor thromboxane from pulmonary intravascular macrophages. The elevation in PAP induced by this reaction is usually clinically insignificant but can

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sometimes be severe and lead to cardiovascular collapse. Additionally, the impact of CPB on the lung function of healthy, young swine, especially CPB of short duration as in our laboratory's model, is unlikely to be as severe as that in a middle-aged to elderly population with underlying cardiac and pulmonary disease and underlying pulmonary hypertension of variable severity. Absence of these factors may lead to underestimation of the potential benefits of the therapies tested. Despite no changes in PAP with inhaled milrinone pre-CPB (and no differences in PAP within or between all groups) in the porcine study discussed earlier, clinical benefits have been demonstrated with pre-CPB inhaled milrinone in high-risk cardiac surgery patients [258]. Patients treated with inhaled milrinone before CPB had lower PAP after CPB and had less emergency reinitiation of CPB after weaning.

The significance of the changes in pulmonary capillary wedge pressure (PCWP) over the course of the experiment is unclear. The sildenafil group had significantly higher PCWP from the time the medication was administered onwards. In the absence of pulmonary venous or left-sided cardiac structural disease, PCWP normally reflects left-sided cardiac filling and may rise in the presence of ventricular dysfunction. Left-sided cardiac filling is itself determined by right ventricular cardiac output, which was measured using the thermodilution technique by the Swan-Ganz catheter and was not statistically different within and between groups. As sildenafil is unknown to have effects on cardiac contractility, it is difficult to explain the etiology of this increase in PCWP. Cardiac filling would be expected to *decrease* with sildenafil-induced systemic vasodilation, and significant changes in cardiac filling are unsupported given the absence of significant changes in central venous

pressure. It is important to remark that the increase in PCWP may have repercussions on the PAP. Without attaining the threshold of venous hypertension, the increase in PCWP noted would require PAP to increase by an equivalent degree in the absence of changes in the transpulmonary gradient. The statistically significant increase of 2 mmHg in mean PAP seen in the sildenafil group may in part be explained by the ~2 mmHg increase in the PCWP.

Oxygenation

There was improved oxygenation in the SN-50 and milrinone groups compared to the sildenafil and placebo groups at 30 minutes post-CPB that was no longer significant at 60 minutes. SN-50, as a transcription factor inhibitor, would not be expected to affect hypoxic vasoconstriction as an intravenous vasodilator would. Simultaneously, by its preventative effects on pulmonary endothelial dysfunction and ischemia-reperfusion, the alveolar-arterial oxygen gradient may be improved versus placebo. The improvement in oxygenation seen with inhaled milrinone is likely due to vasodilatation of pulmonary vessels in the ventilated portions of the lung that receive the drug. Inhaled sildenafil lead to an increase alveolar-arterial oxygen gradient after CPB, consistent with previous studies [274]. In that study, nebulization of sildenafil after CPB prevented the increase in alveolar-arterial gradient but only during the duration of the nebulization, with subsequent increase in the gradient once the nebulization was finished.

Vascular Reactivity

There is impairment of endothelium-dependent relaxation of pulmonary arteries induced by CPB upon reperfusion of the pulmonary vascular bed at weaning [281]. A decrease in endothelium-dependent relaxations after CPB as compared to a non-CPB control has been demonstrated in several studies from our laboratory using this porcine model [231, 255, 274] and suggests the presence of endothelial dysfunction. The placebo group in this study served as a baseline of impaired endothelium-dependent relaxations against which the three active treatment groups could be compared. As in these other studies, there was no difference in the endothelium-independent relaxations in response to the exogenous NO donor SNP, which suggests that the endothelial dysfunction must be attributed to anomalies in the signaling function of the endothelial cells rather than the effector vascular smooth muscle cells.

ACh-induced endothelium-dependent relaxations were slightly improved versus placebo with sildenafil but only at the lower concentration range of ACh. At the maximal relaxations, sildenafil was not different from placebo. Additionally, SN-50 leads to further impairment of relaxation versus placebo and was also worse than sildenafil and milrinone over a range of concentrations. The ACh-related G_i–protein mediated NO pathway has been shown in two animal models to be preferentially damaged after CPB [281, 282]. ACh induces a dose-dependent vasomotor effect dependent on the preexisting vascular tone. Under resting conditions, ACh induces vasoconstriction and vasodilatation under conditions of elevated tone [283]. Given that cGMP, the second messenger of NO, is degraded by the target of sildenafil, animals receiving this drug may have better relaxations as a result of increased

levels of cGMP. A study focusing on sildenafil from our lab also found that ACh-induced relaxations were improved with this treatment [274]. Interestingly, impaired ACh-induced endothelium-dependent relaxations in the pulmonary arteries of cystic fibrosis was associated with increased tissue levels of NF-κB and ET-1 [102, 284]. The attenuation in ACh-induced relaxation due to endotoxin administration in rats in the septic shock model in the latter study was reversed by the NF-κB inhibitor pyrrolidine dithiocarbamate. These studies suggest that NF-κB inhibition would be likely to produce *improved* ACh-induced endothelium-dependent relaxations, contrary to our findings. As NF-κB has been shown to control ACh receptor expression in neuronal tissues, it is possible that it also influences ACh receptor expression in endothelial cells and that NF-κB inhibition leads to unexpected downregulation of these receptors, leading to decreased sensitivity to ACh [285]

BK is an agonist of B_2 receptors coupled to G_q-proteins, which causes release of NO and EDHF from the endothelium but, as described earlier, also stimulates prostacyclin release, causing vasodilatation regardless of the preexisting vascular tone. However, all experiments in this study were performed in presence of the cyclooxygenase inhibitor indomethacin which blocks the endogenous production of prostacyclin such that the focus is on the NO pathway. Thus, all improvements in relaxations are logically a product of increased NO production or increased vascular smooth muscle cell (VSMC) sensitivity to NO.

Improvement in endothelium-dependent relaxations with inhaled sildenafil can be explained by its inhibition of cGMP catabolism, with cGMP being the second

messenger of NO within VSMC that brings about vasodilation. In the context of decreased pulmonary NO production and decreased tissue cGMP seen after CPB [185, 193], sildenafil potentiates the action of the remaining NO and increases cGMP. Likewise, milrinone's inhibition of cAMP catabolism reverses the fall in cAMP seen after CPB [255]. Greater endothelium-dependent relaxations due to sildenafil than milrinone may be explained by the use of indomethacin during our vascular reactivity studies to prevent endogenous prostacyclin production as explained above. Thus, vascular relaxation is forced to occur principally through the NO (and therefore cGMP) pathway than the prostacyclin-stimulated cAMP pathway and consequently be more sensitive to inhibition of cGMP metabolism than inhibition of cAMP metabolism. Additionally, cGMP can itself inhibit PDE-3 [55], which may give sildenafil an indirect inhibitory effect on PDE-3 as well as its direct effect on PDE5.

The mechanisms through which NF-κB inhibition leads to improvement in endothelium-dependent relaxations have not been clearly described. In fact, there is no existing literature describing the effects of NF-κB inhibitors on pulmonary vascular reactivity. However, one of the studies mentioned earlier examined the effects of these drugs on pulmonary hypertension [12]. While vascular reactivity studies were not performed in their paper, its findings permit speculation on the physiologic mechanisms underlying the findings of the present study. Duffy and coauthors found that animals treated with SN-50 exhibited a significantly weaker increase in plasma ET-1 levels as compared to the untreated CPB/DHCA group, as NF-κB has been shown to be a transcription factor for ET-1 [103]. A different study demonstrated the impact of ET-1 on pulmonary vascular reactivity, showing that

the ET-1 receptor antagonist tezosentan could reverse the impairments in BK-induced, but not ACh-induced, endothelium-dependent relaxations caused by CPB [81]. Thus, inhibition of NF-κB by SN-50 may lead to improved BK-induced endothelium-dependent relaxations through its reduction in production of ET-1.

Given the limited pulmonary vascular reactivity data for SN-50, it is difficult to adequately explain its relative efficacy in reversing impairment in endothelium-dependent relaxations compared to sildenafil and milrinone. Considerations include dosage (principally in relation to SN-50) and different routes of administration between SN-50 and the PDE inhibitors. The dose of SN-50 used for this study was based on the paper by Duffy and coauthors [12], which was based on their own preliminary studies and chosen to reduce NF-κB activity in the heart 120 minutes after CPB/DHCA to near baseline levels without abolishing NF-κB activity, and confirmed by quantifiying NF-κB in left ventricular myocardium. This dose may not produce equivalent suppression in animals undergoing a shorter duration of CPB *without DHCA*. Given that a basal level of NF-κB may be necessary to maintain the anti-apoptotic signal in some cell types [286], both over- and under-dosing may lead to suboptimal results. Exploring the effects of SN-50 on NF-κB activity in our laboratory's porcine model of CPB without DHCA as well as the establishing a dose-hemodynamic response curve may be interesting avenues for further research. Unfortunately, constrained resources prevented these analyses from being performed during this study. Finally, differences in route of administration (intravenous for SN-50 vs. inhaled for the PDE inhibitors) could be argued to have played a role in the difference observed. However, this aspect was carefully

considered during development of the study's methodology. SN-50 was limited to intravenous administration as no data exists on the efficacy of inhaled administration of this drug, with all earlier studies using the intravenous route. Additionally, given that SN-50 targets the inflammatory response rather than directly acting on vascular tone, the reduction in systemic vasodilatation obtained by administering PDE inhibitors through inhalation rather than intravenously would not be expected to be seen with SN-50. Inhalation was purposely chosen as the route of administration for sildenafil and milrinone as it was shown in studies mentioned earlier, for both medications, to improve hemodynamics when compared to intravenous administration and because inhalation is quickly becoming the standard mode of administration for PDE inhibitors during clinical cardiac surgery. Given that this study sought to compare SN-50 to the *most effective* current therapies for post-CPB pulmonary hypertension, it was thought that limiting the PDE inhibitors to their intravenous form in order to facilitate comparisons would not be true to the goals of this research.

Limitations

This study did not seek to study the pharmacological effect of the different therapies. Two of the three therapies tested were shown to have pharmacodynamic efficacy in several studies in swine discussed earlier. SN-50 administered intravenously prior to CPB and DHCA lead to decreased NF-κB activity levels in nuclear extracts from LV myocardium 120 minutes after CPB/DHCA compared with CPB/DHCA controls [12]. Heart tissue from SN50-treated animals had NF-κB activity levels slightly lower than control animals that did not undergo CPB/DHCA.

Also, immunoblots of LV myocardial tissue demonstrated that SN50 treatment maintained IκB protein at higher levels than in untreated animals. In the case of the PDE inhibitors milrinone, the pharmacodynamic effect is an increase in intracellular cAMP. Pulmonary artery rings intracellular cAMP was significantly increased with both inhaled and intravenous milrinone compared to the untreated CPB group and the control group [255]. No studies measured pulmonary artery intracellular cGMP but given that the mode of administration was identical to that of inhaled milrinone and that the mode of action has already been clearly established for its oral and intravenous form and is quite similar to milrinone, one can reasonably expect the inhaled sildenafil exerted its effect at the cellular level in this study. Furthermore, two animal studies of inhaled sildenafil showed either clear hemodynamic effects or effects on pulmonary vascular reactivity when sildenafil was inhaled before CPB [274, 287]. There were also no measurements of the pharmacokinetics in plasma or in lung parenchyma of the different drugs used. The doses used were extrapolated from the literature.

As mentioned earlier, the translatability of this model in young and healthy swine to a human population with risk factors for pulmonary hypertension who are undergoing cardiac surgery may be limited. However, experience with inhaled milrinone in this model predicted future clinical success with this therapy years after the original animal study [255, 258]. Furthermore, the good health of the animals and the absence of supplemental provocative factors for pulmonary hypertension beyond CPB, in particular protamine, may mask beneficial clinical effects of the therapies that might be seen when used in a sick human population

that is already at high risk for pulmonary hypertension. This phenomenon may the

source of the inability of this model to consistently produce elevations in PAP in

untreated groups, thus possibly hiding hemodynamic benefits in the treated groups.

CONCLUSION

Endothelium plays a crucial role in the maintenance of vascular integrity and hemostasis and control of vascular tone through the production and release of a number of mediators. Endothelial dysfunction is seen in a number of cardiovascular disorders and may occur after clinical cardiac surgery with cardiopulmonary bypass. While permitting life-saving surgery, cardiopulmonary bypass also submits patients to a potent inflammatory reaction and disturbs the hemostatic system profoundly. Pulmonary endothelial dysfunction occurs as a result of CPB-induced lung ischemia-reperfusion. Prominent players in this effect are free radicals, cytokines, and macrophages and neutrophils in addition to the endothelial cells themselves. Altogether these elements lead to endothelial cell activation and dysfunction and pulmonary vasoconstriction. Clinically, the manifestations may be as severe as post-CPB pulmonary hypertension and subsequent RV failure with cardiogenic shock, which carries a grave prognosis. Prevention is often a better option than treatment after the fact; however, the perfect drug has not yet been found for this indication. The phosphodiesterase inhibitors milrinone and sildenafil are effective as pulmonary vasodilators but their use may be limited by side effects. The novel NF-κB inhibitor SN-50 targets the reaction to CPB at the level of the transcription factor in the hope of limiting pulmonary vasoconstriction and endothelial dysfunction with fewer side effects. In the present study, inhaled sildenafil lead to a statistically significant but clinically unimportant elevation in PAP post-CPB that was not significantly different from the PAPs in the other groups. However, the CPB model of pulmonary endothelial dysfunction in young, healthy swine may not generalize well to a human population at risk for pulmonary

hypertension and undergoing clinical cardiac surgery and true pulmonary hypertension may be difficult to detect. Endothelium-dependent relaxations were most improved with sildenafil over placebo and were also better than with milrinone or SN-50 probably due to direct effects on the second messenger of NO. While SN-50 also produced improvements in bradykinin-induced relaxations, the dosage may not have been optimal for our CPB model.

A number of new directions for research are suggested by this project. NF-κB inhibition lead to improved bradykinin-induced endothelium-dependent relaxations in our porcine model using a dose determined for a longer period of CPB including DHCA. Further study into how NF-κB activity is altered in our porcine model of normothermic CPB rather than CPB with DHCA may help determine the dose of SN-50 that would optimally suppress NF-κB activity while maintaining a minimum basal level. Also, milrinone, which is already used in inhaled form in clinical cardiac surgery, provided inferior relaxations in this study compared to inhaled sildenafil, which is almost used exclusively in oral form. Thus, there may be interest in further studying the benefits of inhaled sildenafil in cardiac surgery in addition to further study in animals of NF-κB inhibition during CPB.

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