Université de Montréal

# Tramadol in the elderly:

## Pharmacokinetic and pharmacodynamic modelling in healthy young and elderly subjects

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Thèse présentée à la Faculté des études supérieures
en vue de l'obtention du grade de
Philosophiæ Doctor (Ph.D.)
en sciences pharmaceutiques
option pharmacologie

January12, 2017

# Résumé

Même si la douleur est très fréquente chez les personnes âgées et que ces dernières sont parmi les plus grands utilisateurs d'analgésiques, les preuves factuelles supportant les décisions médicales sont limitées. Récemment, une revue systématique des essais cliniques portant sur les douleurs aigues au bas du dos a permis de constater que les adultes de plus de 65 ans étaient systématiquement exclus des essais cliniques randomisés en dépit des incitations règlementaires à inclure de tels patients dans ces études. Les données en pharmacocinétique (PK) et pharmacodynamie (PD) concernant les analgésiques chez les patients du troisième âge, particulièrement les personnes âgées de plus de 75 ans, sont rares. Comprendre la relation pharmacocinétique-pharmacodynamique (PK/PD) des médicaments employés pour traiter les conditions qui affectent communément nos ainés est fondamentale pour un traitement optimal leur permettant de conserver une bonne qualité de vie et leur dignité et ce, tout en minimisant les effets secondaires délétères. Le tramadol est un opioïde faible communément employé chez les personnes âgées pour soulager la douleur. Pourtant, il y a peu de données sur sa relation PK/PD chez ces mêmes personnes.

Plusieurs essais cliniques visant à établir l'efficacité d'un médicament, et en particulier les analgésiques, produisent des résultats non concluants ou négatifs; les modèles expérimentaux de douleur offrent l'opportunité de comprendre la PD des analgésiques au moyen d'études de plus petite échelle qui minimisent les circonstances environnementales pouvant introduire un biais. Les analyses PK/PD par approche de population permettent d'optimiser les régimes posologiques et de concevoir des essais cliniques qui prennent en considération les connaissances acquises. Le modèle expérimental de douleur employé dans ce programme de recherche nous donne une façon d'évaluer les différences de tolérance à la douleur entre sujets jeunes et âgés de façon quantitative. L'objectif de cette thèse est de contribuer au savoir en caractérisant la relation PK/PD du tramadol et de son métabolite actif, ODM, chez les patients de 75 ans et plus, afin de déterminer s'il existe des différences reliées à l'âge.

Nous avons conduit une étude PK et PD à répartition aléatoire, contrôlée par placébo, comportant deux périodes en chassé-croisé. Treize sujets âgés de plus de 75 ans ayant une insuffisance rénale légère et 16 sujets âgés entre 18 et 40 ans ont été recrutés. Des échantillons de sang et d'urine ont été recueillis sur une durée de 48 heures post-dose. Un modèle expérimental de douleur à base de stimulation électrique a été employé pour évaluer le seuil de tolérance à la douleur (PTT), soit l'intensité maximale qu'un sujet est en mesure de tolérer et ce, employant un stimulus douloureux mais non blessant appliqué au doigt non dominant. Le PTT a été testé à des fréquences de 250 et 5 Hertz et ce, à 17 moments sur une période de 30 heures post-dose.

Une analyse PK noncompartimentale (NCA) approfondie des concentrations plasmatiques et urinaires du (+) et (-) tramadol et du (+)- et (-)-ODM de même qu'une analyse PK par approche de population du tramadol ont d'abord été exécutées. Ces analyses ont démontré que l'exposition générale au tramadol chez les patients âgés est comparable à celle des plus jeunes. Aucunes différences dans les processus d'absorption n'ont été observées. Cependant, une différence significative a été observée au niveau de la demi-vie d'élimination du tramadol chez les personnes âgées, probablement à cause d'une augmentation de sa distribution corporelle. Les différences les plus notables se situent au niveau de la PK de l'(+)-ODM, le métabolite ayant une activité opioïde. Ses concentrations plasmatiques maximales ont été observées plus tard et ont décru plus lentement chez les personnes âgées que chez les jeunes. L'exposition à l' (+)-ODM était significativement plus grande chez les sujets âgés, et tant la clairance rénale que la clairance corporelle totale étaient plus lentes. L'analyse PK populationnelle a confirmé ces observations et identifié qu'une distribution supérieure de même qu'une élimination moyenne de 50% plus longue pour le tramadol chez les sujets âgés. Il est important de souligner que, dans notre groupe de personnes âgées, l'insuffisance rénale était plus fréquente que l'insuffisance hépatique.

Par la suite, avant de procéder à l'analyse populationnelle pour établir une relation entre les concentrations de l'ODM et les seuils de tolérance à la douleur, nous avons analysé les données pharmacodynamiques sous les périodes placébo et tramadol afin de valider le nouveau modèle expérimental de douleur proposé. Nous souhaitions sélectionner le stimulus électrique (5 Hz ou 250 Hz) qui soit le plus sensible pour détecter un changement au niveau de

hla tolérance à la douleur. Tant les jeunes sujets que les plus âgés ont démontré des valeurs de base similaires pour le seuil de tolérance à la douleur et ce, aux deux fréquences sous administration active et placébo. Chez les personnes âgées, la valeur maximale du PTT était de 30% supérieure sous tramadol comparativement au placébo et ce, tant à 5 Hz que 250 Hz; toutefois, la réponse était plus variable pour la dernière fréquence. La tolérance à la douleur, telle que mesurée par la surface sous la courbe de l'effet en fonction du temps (AUEC) sur une période de 24 heures, était significativement plus élevée (au-delà de 160%) chez les personnes âgées pendant le traitement actif comparativement au placebo pour les deux fréquences de stimulation; toutefois, aucune différence significative au niveau de la tolérance n'a été observée chez les plus jeunes. Nous avons émis l'hypothèse que cette différence pouvait résulter de la plus grande exposition des sujets âgés à l' (+)-ODM. Par conséquent, une analyse PK/PD devenait nécessaire pour déterminer si ces changements au niveau du seuil de tolérance à la douleur chez les personnes âgées étaient reliés à une plus grande exposition à l'(+)-ODM.

Finalement, en utilisant des concentrations plasmatiques de (+)-ODM et les données PTT obtenues avec le stimulus de 5 Hz, nous avons conduit une analyse populationnelle exploratoire pour déterminer tout effet de l'âge sur la relation entre les concentrations plasmatiques de (+)-ODM et la tolérance à la douleur. En dépit de valeurs de base semblables pour la tolérance à la douleur, l'effet maximal possible relié au traitement était de 15% supérieur chez les sujets âgés, ce qui pourrait s'expliquer par une exposition plus élevée au métabolite actif, confirmant son mécanisme d'action opioïde. La concentration plasmatique associée à 50% de l'effet maximal n'était pas différente chez le sujet jeune et âgé, indiquant que l'âge n'est pas associé avec une plus grande sensibilité à l' (+)-ODM.

En conclusion, ceci est le premier programme de recherche ayant étudié extensivement la PK et PD du tramadol chez les patients de 75 ans et plus. La valeur de ce programme de recherche va au-delà d'une meilleure compréhension de la PK du tramadol, en améliorant notre compréhension des contributions relatives des clairances rénale et totale au niveau des

changements survenant avec l'âge pour la PK du tramadol et de son métabolite actif chez les personnes âgées en relativement bonne santé. Ce programme contribue également au développement de modèle permettant d'effectuer davantage de recherches chez les personnes âgées puisqu'il est le premier modèle PK/PD populationnel de (+)-ODM chez les sujets de 75 ans et plus. Nos analyses démontrent que les changements reliés à l'âge dans la clairance rénale peuvent résulter en un accroissement proportionnel de l'exposition à l'ODM, et pourraient expliquer les observations faites par certains cliniciens dans la littérature qui rapportent une augmentation des effets (secondaires) à des doses équivalentes chez les personnes âgées. Ceci est d'autant plus de pertinence clinique que l'efficacité et les effets secondaires du tramadol découlant de sa nature opiacée, notamment la sédation, sont principalement reliés à l'(+)-ODM et le seraient davantage chez des patients âgés fragilisés souffrant d'une insuffisance rénale plus prononcée que celle des sujets étudiés au cours de notre recherche.

**Mots-clés** : Tramadol, O-desmethyltramadol, énantiomère, analyse non compartimentale, analyse populationnel pharmacocinétique, analyse populationnelle pharmacodynamique, PK/PD, seuil de tolérance de douleur, personnes âgées, Gériatrique, douleur

# Abstract

Although pain is highly prevalent among the elderly and they are amongst the highest users of analgesics, research to support evidence based treatment decisions is limited. Recently a systematic review of clinical trials in low back pain found that elderly adults older than 65 were systematically excluded from randomised clinical trials despite calls to include elderly subjects in such studies. Pharmacokinetic (PK) and Pharmacodynamic (PD) data on analgesics in elderly patients, especially those older than 75 years, is sparse. Understanding the pharmacokinetic/pharmacodynamic relationship (PK/PD) of medicines used to treat conditions that commonly affect elderly people is key to treating them effectively, allowing them to live with quality of life and dignity and minimising the side effects that can interfere with this. Tramadol is a weak opioid commonly used in elderly patients for pain relief. Yet there is little data on its PK/PD in the elderly.

Many later phase clinical trials, especially in analgesics produce inconclusive or negative results; experimental pain models offer the opportunity to understand the PD of analgesics on a smaller scale and minimise confounding environmental circumstances. Population PK/PD analyses of early research data permit the optimisation of dosing regimens and of the design of phase III clinical trials by taking into account what is learned. The pain model utilised in this research program gives us a way to look at the differences in pain tolerance between young and elderly in a quantitative fashion. The objective of this thesis is to contribute to the knowledge about age-related differences in the PK/PD of tramadol and its active metabolite O-desmethyltramadol (ODM) in subjects 75 years and older in order to examine whether there are age-related differences.

We conducted a double-blind randomised, placebo-controlled, two-period crossover study including 13 elderly subjects (≥75 years) with mild renal insufficiency and 16 young (18-40 years) subjects. Blood samples and urine were collected for 48 hours post-dose. An electrically stimulated pain model (ESPM) was used to test pain tolerance threshold (PTT), the maximum intensity a subject is willing to tolerate, using a painful but non-injuring electrical stimulus applied to the non-dominant middle finger. PTT was tested at both 250 and 5 Hz at each of 17 time-points over 30 hours after a 200 mg dose of extended release tramadol .

An in depth noncompartmental analysis of the PK of (+)- and (-)-tramadol and (+)- and (-)-ODM plasma and urine concentrations as well as a population PK analysis of tramadol were performed. Maximum plasma concentrations of (+)-ODM, the active metabolite, occurred later and plasma concentrations declined more slowly in the elderly than in young subjects. These analyses showed that overall exposure to tramadol in elderly subjects is comparable to that in young subjects. No differences in absorption processes were observed. However, there was a significant difference in tramadol elimination half-life, most probably due to increased distribution in elderly subjects. The most remarkable differences were in the PK of (+)-ODM, the metabolite with opioid activity. Exposure to ODM was significantly greater in elderly subjects and both renal and overall clearance from the body were slower. The population PK analysis supported our findings and identified that a higher distribution and a 50% longer mean elimination half-life was associated with age of 75 or older. A key observation was that in our study population renal insufficiency was more prevalent in the elderly subjects than hepatic insufficiency.

Subsequently, in preparation for a population analysis of the PK and pain tolerance effect of tramadol's active metabolite, (+)-ODM, we analysed pain tolerance data under placebo and tramadol administration to validate the exploratory experimental pain model that we used. We wanted to select the electrical stimulus (5 Hz or 250 Hz) that was most sensitive to detect changes in pain tolerance. Young and elderly subjects showed similar baseline pain tolerance at both 5 Hz and 250 Hz before administration of active and placebo, suggesting that pain tolerance is similar in either frequency. In the elderly, the peak pain tolerance was 30% greater for both 5 and 250 Hz after administration of tramadol as compared to placebo, but the response was noisier for the last frequency. The net pain tolerance over the 24 hours, as measured by area under the effect-time curve (AUEC) during active treatment was significantly higher (over 160%) compared to placebo for both 5 and 250 Hz stimulations in the elderly but no significant difference was observed in the young. We hypothesised that this difference might be due to the higher exposure of elderly subjects to ODM. And therefore, a PK/PD analysis was required to determine whether these age-related changes were due to

altered sensitivity in elderly subjects to PTT or to a greater exposure to the active (+)-ODM metabolite.

Finally utilising plasma concentrations of (+)-ODM and the PTT data from the 5 Hz stimulus, we conducted an exploratory population analysis to determine any age-related effects on the relationship between (+)-ODM concentrations and pain tolerance threshold. Although pain tolerance was similar between young and elderly subjects at baseline, there was a 15% higher maximum possible treatment-related effect that may be associated with the higher systemic exposure to ODM., the active metabolite, thereby confirming its opioid mechanism of action. The concentration at which 50% of effect was achieved was not reduced between the young and elderly, indicating that age was not associated with greater sensitivity to (+)-ODM.

**In conclusion,** this is the first research program to extensively report the PK and PD of tramadol in subjects 75 and older. The value of this research program goes beyond that of a better understanding of the PK of tramadol, by delineating the relative contribution of renal clearance versus overall clearance to age-related alterations in the PK of tramadol and ODM in generally healthy elderly people. This research program also contributes to the development of population models to support further research in the elderly being the first population PK/PD model developed for (+)-ODM in subjects 75 and older. Our findings show that age-related changes in renal clearance versus overall clearance can result in a proportional increase in ODM exposure, and may explain the observation of some clinicians and literature that there is increased side effects at equivalent doses in the elderly. This is potentially of clinical significance since opioid-related efficacy and side effects of tramadol, among them sedation, are primarily linked to (+)-ODM and the risk of side effects would likely be greater in frail elderly subjects with greater renal impairment than those studied in our research.

**Keywords** : Tramadol, O-desmethyltramadol, enantiomer, non-compartmental analysis, population pharmacokinetics, population pharmacodynamics, PK/PD, pain tolerance threshold, elderly, geriatric, pain

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# Liste des abréviations et sigles

| | |
|---|---|
| $\lambda_z$ | Terminal elimination rate constant |
| Aβ | Alpha-beta fibre |
| Aδ | Alpha-delta fibre |
| β-EP | β-endorphin |
| ε | Epsilon |
| θ | Theta |
| $\sigma^2$ | Sigma squared |
| $t_{1/2}$ | Half-life |
| $T_{max}$ | Time to maximum concentration |
| 5-HT | Serotonin |
| AAG | Alpha 1-acid glycoprotein |
| ACH | Acetylcholine |
| AND | Adenosine |
| AIC | Aikaike information criteria |
| AUC | Area under the curve |
| $AUC_{0-\infty}$ | Area under the plasma-concentration-time curve from 0 to infinity |
| AUEC | Area under the effect-time curve |
| AUMC | Area under the curve for the first moment |
| ATP | Adenosine triphopshate |
| BIC | Bayesian information criteria |
| BLQ | Below the limit of quantification |
| BSV | Between subject variability |
| C | Concentration of drug |
| $CA^{2+}$ | Calcium ion |
| cAMP | CyclicAMP |
| CB | Cannabinoids |
| CCK | Cholecystokinin |
| CGRP | Calcitonin gene-related peptide |

| | |
|---|---|
| CL | Clearance |
| $CL_{tot}$ | Total plasma clearance |
| $C_{last}$ | Last plasma concentration |
| $C_{max}$ | Maximum plasma concentration |
| CNS | Central nervous system |
| CPM | Central pain modulation (previously DNIC) |
| CPT | Current perception threshold |
| CV% | Percentage of coefficient of variation |
| CYP | Cytochrome P |
| D | Deviation |
| DAMGO | D-Ala$^2$,N-methyl-Phe$^4$,Gly$^5$-ol |
| DA | Dopamine |
| DF | Descending facilitatory |
| DI | Descending inhibitory |
| DH | Dorsal horn |
| DNIC | Diffuse noxious inhibitory controls (now known as CPM) |
| DNP | Descending inhibitory pathways |
| DP | Descending pathway |
| DPN | Descending pathways neuron |
| DRG | Dorsal root ganglia |
| DRT | Dorsal reticular nucleus |
| DYN | Dynorphin |
| E | Effect |
| ECG | Electrocardiogram |
| EM | Endomorphin |
| EMA | European medicines agency |
| ENK | Encephalin |
| EPS | Epsilon |
| ESPM | Electrically stimulated pain model |
| EXIN | Excitatory interneurons |

| | |
|---|---|
| F | Bioavailability |
| FOCE | First-order conditional estimation |
| GABA | Gamma-hydroxy-butyric acid |
| GAL | Galanin |
| GFR | Glomerular filtration rate |
| GLU | Glutamate |
| GLY | Glycine |
| GPCR | G-protein-coupled receptors |
| h | Hour |
| Hz | Hertz |
| HIST | Histamine |
| IA | Intra-articular |
| IIV | Inter-individual variability |
| IML | Intermediolateral cell column |
| IN | Interneurons |
| ININ | Inhibitory interneurone |
| IR | Immediate release |
| I.V. | Intravenous |
| $k_a$ | Rate constant of absorption |
| $k_e$ | Excretion rate constant |
| $k_{el}$ | Rate constant of elimination |
| $k_m$ | Metabolism rate constant |
| K+ | Potassium ion |
| L | litre |
| LRT | Likelihood ratio test |
| MC | Melanocortin |
| MIT | Mean input time |
| MN | Motoneurones |
| MRT | Mean residence time |
| m/s | Metres per second |

| | |
|---|---|
| $M_u$ | Metabolite in urine |
| NA | Not applicable |
| NA | Noradrenaline |
| NCA | Non compartmental analysis |
| NCG | Nucleus cuneiformis |
| NGF | Nerve growth factor |
| NMDA | N-methyl-D-aspartate |
| NO | Nitric oxide |
| NP | Not provided |
| NPFF | NeuropeptideFF (FLFQPQRFa) |
| NS | Nociceptive-specific |
| NT | Neurotransmitter |
| NTS | Nucleus tractus solitaris |
| $OBS_{conc}$ | Observed concentration |
| OFQ | OrphaninFQ (nociceptin) |
| OFV | Objective function value |
| ODM | *O*-desmethyltramadol |
| ORL 1 | Opioid receptor-like 1 |
| OT | Oxytocin |
| PAF | Primary afferent neuron |
| PAG | Peri acqueductal grey matter |
| PBN | Parabrachial nucleus |
| PD | Pharmacodynamic |
| PFC | Prefrontal cortex |
| PG | Prostaglandin |
| PK | Pharmacokinetics |
| PN | Projection neuron |
| popPK | Population pharmacokinetics |
| PPT | Pain perception threshold |
| PreG | Preganglionic |

| | |
|---|---|
| PTT | Pain tolerance threshold |
| RM | Raphe magnus |
| RUV | Residual unexplained variability |
| RVM | Rostro ventromedial medulla |
| SD | Standard deviation |
| SP | Substance P |
| SPID | Sum of pain intensity differences |
| S1 | Primary somatosensory cortex |
| S2 | Secondary somatosensory cortex |
| SR | Sustained release |
| $T_0$ | Time zero |
| TOAD | Tramadol extended release formulation intended for once daily dosing |
| U | Drug in urine |
| VAS | Visual analogue scale |
| $Vd_\beta$ | Volume of distribution |
| $Vd_\beta/f$ | Apparent volume of distribution |
| $Vd_{SS}$ | Volume of distribution at steady state |
| VP | Vasopressin |
| VPC | Visual predictive checks |
| WDR | Wide dynamic range neurons |
| WRES | Weighted residuals |

*To my Mom, who planted the dream of being a scientist when little girls weren't encouraged to dream in that direction, who always challenged me to be curious and to never quit. To my sister who has taken up that torch. To my dear friends: Sylvie Bouchard, who gave me confidence in my ideas and intelligence and Annie Gingras, who helped me persevere and do it with a sense of humour, without their example as intelligent strong women and their encouragement and support this would not have been possible. To my husband, and children who were always there for me, encouraging me on this journey. Thank you!*

# Remerciements

I would like to thank my director of research, France Varin for her patience and courage in taking on an adult student. Her knowledge and experience and willingness to share it have been integral to my learning and this research.

I would like to thank my co-director, Mohamad-Samer Mouksassi, for sharing his precious time and passion and knowledge of pharmacometrics.

I would like to thank the members of my jury who have graciously given their time to evaluate my research and given their invaluable advice to improve my thesis.

I would like to thank my former work colleagues, specifically Sylvie Bouchard, Annie Gingras, David Karhu, Caroline Fradette and James Howard-Tripp for their support of me as a student and researcher.

I would like to thank my former and current lab colleagues, Chen Chun Lin, Anne Nguyen, François Gaudreault, Paul Gavra and Fady Thomas for teaching me, challenging me, supporting me and helping me to laugh.

Finally I would like to thank my husband, my children, my Mom, my sister and my dear friends who encouraged me to keep going and supported me in doing so.

# Section 1 : Introduction

The proportion and absolute number of elderly people in populations around the globe are increasing because of decreased mortality in infants and young people and increased life expectancy in the elderly. It is forecasted that by 2025 life expectancy will be between 60-80 years in all regions of the world (1). Even in regions such as Africa which currently has many countries with relatively young populations, it is expected that the aging of the population, represented as a threshold of 20% elderly, will be attained at a much faster rate than in countries like France and the UK, that have currently achieved that proportion. This global demographic shift requires a better understanding and treatment of many of the health concerns that elderly persons experience, in order to ensure that individuals have the best possibility for good quality of life as they age. Furthermore, it represents a challenge for societies, particularly in countries with less economic means, to maintain health and social systems. Despite this, research on medicinal treatments used in elderly patients is lacking. A search of the Clinicaltrials.gov data base revealed that in 2010, of the 1545 clinical trials conducted in central nervous system (CNS) indications, only 1.5% included patients older than 75 years. Furthermore, less than 10% of drug delivery technology trials conducted included Pharmacokinetic (PK) assessments in the elderly (2, 3).

Older adults are at higher risk both for acute and chronic pain (4). The prevalence of pain increases up to the seventh decade of life and may be as high as 50% of persons in the community setting and 80% of persons in residential care facilities (5-7). Pain in the elderly may arise from a variety of sources, with back and neck pain and osteoarthritis being globally amongst the top ten health conditions associated with disability in populations 60 years and older. Furthermore, pain experienced by the elderly is often moderate to severe in intensity. Analyses of data from the 2008 cross-sectional, National Health and Wellness Survey in 5 European countries (France, Germany, Italy, Spain and the UK) revealed that in persons over 60 years of age reporting pain within the month prior to the survey, intensity was severe or moderate in a proportion of 24% and 63%, respectively (8). The natural adaptive response of limiting activity due to acute pain, can become maladaptive in the situation of persistent pain in elderly persons where limiting activity can exacerbate age related decrease in range of

motion, muscle strength and tone and increase in weight, all of which can lead to greater pain. Furthermore, the older person may also restrict social interaction, an important factor in successful aging (9, 10). The complexity of using analgesics in elderly persons cannot be underestimated, key considerations that affect PK and Pharmacodynamics (PD) include age-associated changes in body composition and function. This is particularly important in the presence of frailty and impaired cognition and must take into account the heterogeneity of the expression of these traits of aging in individuals, some of whom may remain relatively healthy into the last decades of life while others experience impairments earlier (11).

A conventional definition of "elderly" is chronological age of 65 years old or older, while individuals 65 through 74 years old are referred to as "early elderly" and those over 75 years old as late elderly (12). Others have identified that chronological age is not a reliable way to identify elderly persons at risk. Instead they propose that a phenotype of frailty is a better marker for risk in the elderly. Frailty is theoretically defined as a clinically recognizable state of increased vulnerability resulting from aging-associated decline in reserve and function across multiple physiologic systems such that the ability to cope with everyday or acute stressors is comprised (13). To meet an operational definition by Fried et al. (14) the elderly person must meet three out of five criteria: low grip strength, low energy, slowed walking speed, low physical activity, and/or unintentional weight loss. In a recent review of the definition of elderly in 20 clinical practice guidelines, Singh and Bajorek (11) found that 3 clinical guidelines define elderly based on chronological age and the remaining 17 provide no definition. They indicate that representation of 'elderly' in guidelines needs to be less based on chronological age or generic definitions rather they should establish a direct link between an individual patient's characteristics and the pharmacology of their prescribed medication.

Good pain treatment in the elderly must be based on sound understanding of the circumstances of aging, including the presence of comorbidities, polypharmacy and variability in the aging process and PK and PD of medications (10). Yet PK and PD data on analgesics in elderly patients, especially those aged >75 years, are sparse (1-3, 10). Standard pain treatments must be studied to determine the impact of age related changes on PK and PD, particularly with regard to analgesic efficacy and the effect of co-morbid diseases and concomitant medications (15).

# Chapter 1 : Fundamental and clinical aspects of pain and aging

## 1.1 Anatomy and physiology of pain systems

The nociceptive system is a dynamic system that undergoes plastic changes and is a result of the modulation of afferent activity via peripheral and central mechanisms (12). Understanding this system requires knowledge of its physiology as well as molecular and behavioral pharmacology. Perception of and reaction to painful stimuli requires the interaction of a series of complex mechanisms: reception of noxious stimuli, transmission of information about those noxious stimuli from the periphery to central nervous system (CNS), perception and reaction in the higher centres and modulation of the pain signal.

### 1.1.1 Anatomy of the pain system

The cells of the pain system can be divided into four main categories: primary afferent neurons (PAF), projection neurons (PN), interneurons (IN) and neurons of the descending pathways (DPN) (Figure 1).

***Primary Afferent Neurons***

Primary afferent neurons (PAF) terminate in free nerve endings known as nociceptors that are found in the skin, muscles, joints and viscera. Two types of PAF are associated with these nociceptors namely Aδ and C fibres (Figure 1). Aδ nociceptors are responsible for the sensation of sharp, acute pain and respond to mechanical and thermal nociception and while C nociceptors are responsible for the sensation of slow burning pain from mechanical, thermal and chemical stimuli and constitute the majority of nociceptors. Aδ nociceptors, which are larger myelinated fibres of 1-5 μm diameter, rapidly transmit nociceptive stimuli at 5-30 meters/second (m/s). Aδ nociceptors are mainly specialized to detect dangerous mechanical and thermal stimuli and trigger a rapid response. C fibres, which are unmyelinated and smaller (0.2 to 1.5 μm) in diameter result in a slower transmission of signals (0.5-2 m/s), respond to strong mechanical, thermal and chemical stimuli and are the most ubiquitous. Some are

specialized to detect single sensations such as pinch or heat but most are polymodal. PAFs run from the peripheral site of injury primarily to the I and II laminae of the dorsal horn (DH) of the spinal cord.

***Projection Neurons***

Projection neurons (PN) synapse with the PAF in the DH of the spinal cord and project to the thalamus, hypothalamus, nucleus tractus solaris (NTS), parabrachial nucleus (PBN), periaqueductal grey matter (PAG) and amygdala (Figure 1). PNs can either transmit only nociceptive information or they can be non-specific receiving both nociceptive information from Aδ and C fibres and other sensory information from Aβ fibres (sensory neurons that detect light touch) and these non-specific PN are known as wide dynamic range (WDR) neurons.

The PN decussate in the DH before ascending in the contralateral spinal tract. There are two primary tracts through which this secondary neuron may ascend:

- The spinothalamic tract is important in the localisation of pain. Secondary neurons that follow this tract synapse with a third neuron within the thalamus; this third neuron then ascends and terminates in the somatosensory cortex.
- The spinoreticular tract is important in the emotional aspects of pain; it ascends to the reticular formation of the brainstem before passing through the thalamus and hypothalamus and making many further projections into the cortex.

As the PN passes through the PAG and raphe magnus in the Rostrovental Medulla (RVM), it makes a variety of synaptic contacts that have important functions in the modulation of pain. PAF, IN and DPN interact to determine the activity of the PN (13).

***Interneurons***

Interneurons (IN) are located in the DH of the spinal cord and brainstem (PAG, RVM). They can act as inhibitory interneurons (ININ), also called OFF cells, or as excitatory interneurons (EXIN), acting pro-nociceptively or anti-nociceptively, respectively (Figure 1).

***Neurons of the descending pathways***

Neurons of the descending inhibitory pathways (DNP) can be part of the descending facilitatory pathway (DF) or the descending inhibitory (DI) pathways (Figure 1). These neurons originate in the RVM and other brainstem nuclei descending to the DH where they interact with the PAF, IN and PN as well as pre-ganglionic neurons of the sympathetic system and motorneurons (MN).

## 1.1.2 Initiation of the pain system response to noxious stimuli

Thermal, chemical or mechanical noxious stimuli result in the activation of mast cells close to nociceptors leading to the release of inflammatory mediators (e.g. histamine, nerve growth factor (NGF), bradykinin and prostaglandin). Binding of these inflammatory mediators to receptors such as G protein-coupled receptors (GPCR) and Tyrosine Kinase A receptors in the cell membrane of the nociceptor leads to activation of the primary afferent fibre by means of propagation of a graded action potential (14).

The signal transmitted from the nociceptor is processed within the brain. As stated earlier, the somatosensory cortex is key to the localisation of pain. However, other areas including the primary and secondary somatosensory cortex (S1 and S2), the insulae, the anterior cingulate, prefrontal cortex and thalamus are also important in pain perception and emotional and physical response.

Pain modulation is another important mechanism in the perception of and response to pain. In general, four regions of the CNS are involved in pain modulation (12):

1) Segmental signal inhibition which involves the inhibition of pain by IN in the DH of the spinal cord
2) Conditioned pain modulation (CPM (previously DNIC)) which uses heterotopic stimulation to reduce the intensity of perception of pain
3) Inhibition through the brainstem network in the PAG and RVM that modulate pain transmission through pronociceptive (ON) and antinociceptive (OFF) cells.
4) Cognitive and affective cortical centres appear to exert a top-down control in pain modulation.

A neuroimaging study by Hadjipavlou et al. (15) found an anatomical link between descending inhibition from higher centres of the brain, such as the prefrontal cortex (PFC) , amygdala, thalamus and hypothalamus, to the descending pain modulatory system in the PAG, the RVM and the Nucleus Cuneiformis (NCG). These higher centres may play an important role in the response to pain. The amygdala is posited to affect the uncertainty associated with pain and fear and therefore to allow humans to plan antinociceptive strategies. Opioid-induced hypoalgesia in the amygdala, PAG and RVM suggests, in turn, that these three regions are involved in planning and mediating antinociception. Descending inhibitory neurons from these higher centres project to IN and secondary neurons in the DH of the spinal cord to inhibit or enhance pain transmission. (16-22).

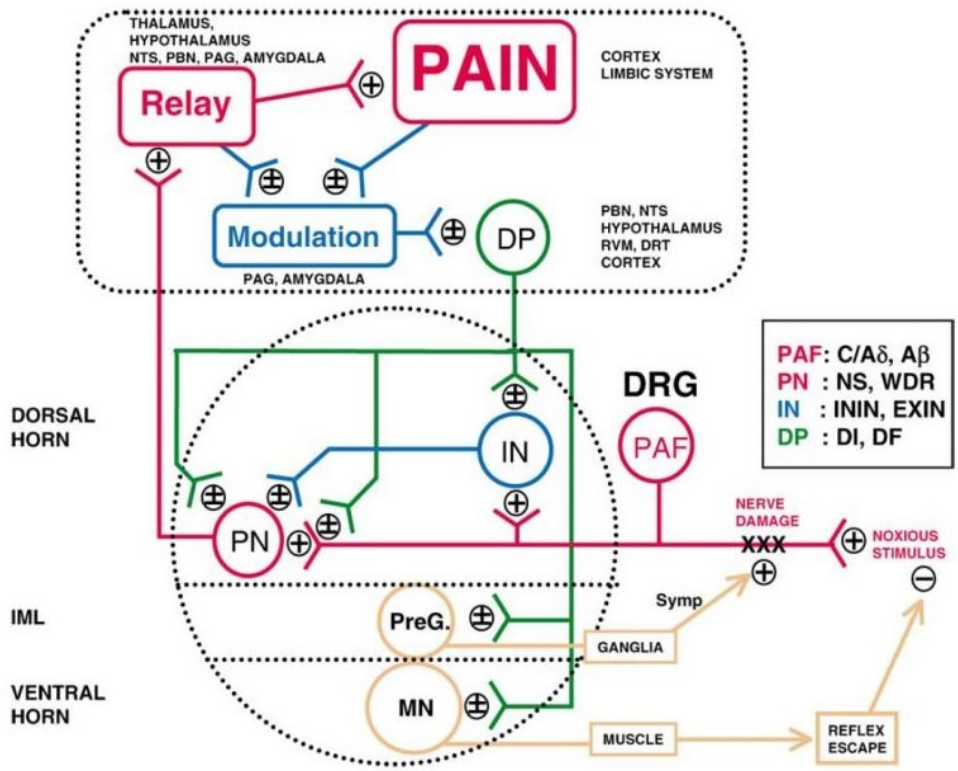


Figure 1. Ascending and descending inhibitory pain pathways

Aβ – Alpha-beta fibre; Cfibre – C fibre; Aδ– Alpha-delta fibre; EXIN – excitatory interneurons; DP –descending pathway; DRG – dorsal root ganglia; DRT – dorsal reticular nucleus; IML – intermediolateral cell column; IN – interneurons; ININ inhibitory interneuron; MN – motoneurons; NS – nociceptive-specific; NTS – nucleus tractus solitaries; PAF – primary afferent fibre; PAG – periaqueductal grey; PBN – parabrachial nucleus; PN – projection neurons; PreG – preganglionic; RVM – rostroventral medulla; WDR – wide dynamic range (16)

Reprinted from Prog Neurobiol.; 66(6): 355-474, 2002. Millan MJ. Descending control of pain (16) with permission of Elsevier. Whether the descending inhibitory neurons inhibit or enhance pain transmission is governed by a series of neurotransmitters, amongst them are monoamines, noradrenaline and serotonin (Figure 2) (16). Opioid receptors are highly expressed in descending modulatory pathways including RVM and PAG and activation of opioid receptors in these locations directly inhibits pain transmission in the spinal cord (13).

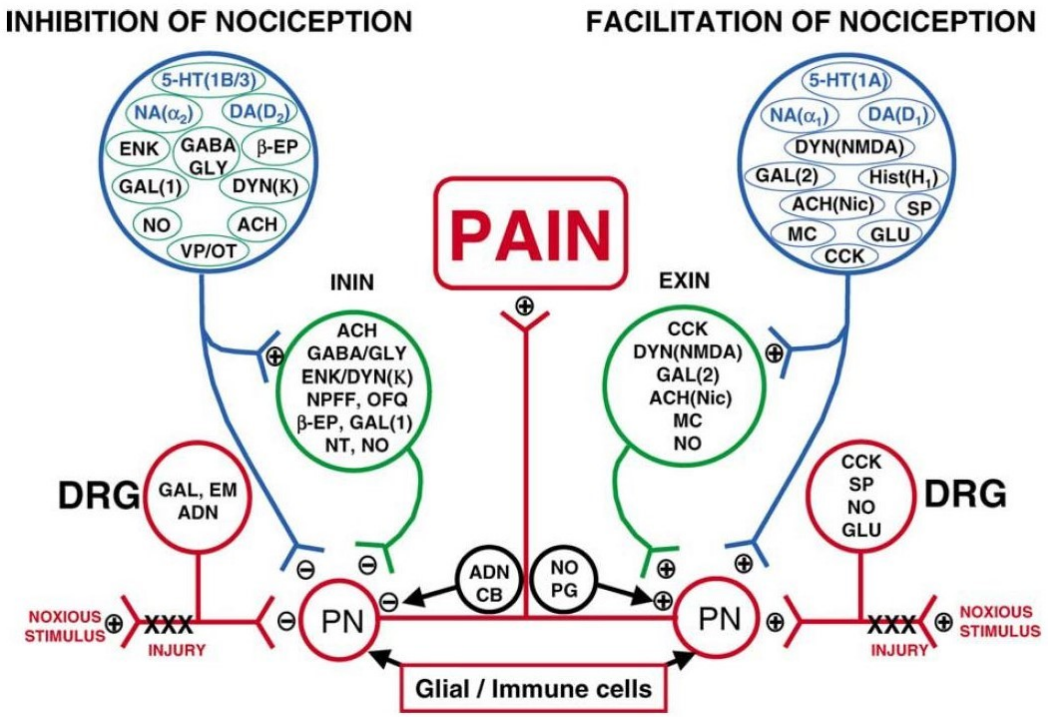


Figure 2. Neurotransmitter inhibition or enhancement of pain transmission

β-EP – β-endorphin; 5-HT – serotonin; ACH –acetylcholine; ADN – adenosine; CB – cannabinoids; CCK –cholecystokinin; CGRP – calcitonin gene related peptide; DA – dopamine; DRG – dorsal root ganglion; DYN – dynorphin; EM – endomorphin; ENK – encephalin; EXIN - excitatory interneuron; GABA – γ-hydroxy-butyric acid; GAL – galanin; GLU – glutamate; GLY – glycine; HIST – histamine; ININ - inhibitory interneuron; MC – melanocortin; NA – noradrenaline; NMDA – N-methyl-D-aspartate; NO – nitric oxide; NPFF – neuropeptideFF; NT-neurotransmitter ; OFQ – orphaninFQ (nociceptin); OT – oxytocin; PG – Prostaglandin; PN – projection neuron; SP – substance P; VP - vasopressin

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## 1.2 Age related changes in the pain system

As referenced earlier, the prevalence of pain especially chronic pain increases from middle age onwards. Given the general trend to age related sensory decline in other sensory systems, such as the taste, auditory and visual systems, it would be surprising that there is no similar trend in the sensory capacity of the pain system (17, 18). It is generally held that the threshold for perception of painful stimuli also named presbyalgos is increased while pain tolerance threshold (PTT) is decreased in elderly subjects and patients (9, 17, 19). Research in both animal models and humans has attempted to elucidate the basis for age related changes in the perception of and response to pain.

Reviews of the preclinical literature on age deficiencies in nociception and pain behavior (18, 20) found that beginning at midlife, changes in neuroanatomy, neurochemistry and pain modulatory systems may be associated with alterations in sensitivity. The conclusion of this review was that, in rats:

- Reflexive responses to painful stimuli were not changed with age; although it may take longer for older animals to undertake complex avoidance behaviors
- Increased sensitivity to tonic pain starting at mid-life may be the result of a reduction in the size and number of neurons in the dorsal root ganglia and degeneration of neural inhibitory system

Relevant to our study, Hoskins et al. (21) found that, in rats, there is a loss in efficacy of spinally administered opioids and subsequent research indicated that, although the density of μ-opioid receptors was not decreased, there was a reduced affinity of [D-Ala$^2$,N-methyl-Phe$^4$,Gly$^5$-ol]enkephalin (DAMGO), a u-opioid receptor agonist, in elderly rats as compared to young or mature rats.

Studies in humans, in general, have drawn inconsistent conclusions with regard to the purported increase in PPT and decrease in PTT in the elderly (22). In experimental studies, the modality of the painful stimulus seems to play a key role. PPT has been shown to decrease with thermally induced pain (23-26) but to increase following mechanically induced pain (27, 28). Results of published studies on age related changes in PTT using electrical nociceptive

stimuli are less clear with one demonstrating a no change (29) and two demonstrating reduced PTT (30, 31). Perception of painful stimuli may be affected by age related effects on peripheral nociceptors. After thermal noxious stimuli, myelinated A-δ fibres showed reduced pain perception and longer sensory evoked potentials while both parameters remained unchanged for unmyelinated C-fibres. This apparent discrepancy is possibly due to reduced density and function of myelinated fibres, including structural modification and reduced conduction velocity with age (32, 33). Tseng et al. (34) found a reduction in the sensory areas of the brain activated and the magnitude of the activation in the elderly using functional magnetic resonance imaging after noxious thermal stimulation.

Changes in pain modulation mechanisms affect both opioid and non-opioid mechanisms and may play an important role in differences seen in pain tolerance. Evidence has been demonstrated for age related reduced pain-modulatory capacity with regard to central pain modulation (CPM )(35, 36). This research suggested that CPM effects resulted in a higher tolerance to heterotopic cold pain in young subjects and pain ratings associated with the cold stimulus were higher in elderly subjects. Thus, in the elderly increased sensitivity of WDR neurons to noxious stimulation resulting from deterioration of CPM mechanisms could result in a net increase in perceived pain. Clinically, this could explain the lower pain tolerance seen in elderly patients and the increasing prevalence of chronic pain conditions. Moreover, there appears to be differences in neuroplasticity in elderly persons: temporal summation occurring more readily, resulting in heightened sensitivity and heightened risk of the occurrence of chronic pain with age (27).

## 1.3 Age related changes in pharmacokinetics

The pharmacokinetics of many drugs are altered in the elderly (37). Alterations in organ function, body composition, concomitant medications and the higher risk of co-morbid diseases all play a part in these differences (38). These changes can affect absorption, distribution, metabolism and elimination. Pharmacokinetics of medicines in the elderly, particularly with older analgesics is not well documented (1-3).

***Absorption***

For orally administered medications, increases in the gastric emptying time and decrease in peristalsis can result in slower transit through the gastrointestinal system altering the time during which the system is exposed to the drug and can absorb it. Gastric pH is increased, decreasing gastric dissolution of basic medications and decreasing absorption of acidic medications. On the other hand, higher content of mucosal connective tissue and reduced mesenteric blood flow along with atrophy of the macro and microvilli result in reduced ability of the system to absorb medications. As a result, many medications have altered bioavailability in the elderly (37).

***Distribution***

Differences in body composition, rate of blood flow and changes in binding of medications to plasma proteins, fatty tissue and other biologic matter which can lead to medications being distributed differently in the bodies of healthy elderly persons. Many of the co-morbid diseases for which elderly have greater risk can further affect distribution.

Elderly persons have a lower lean body mass and higher ratio of fatty tissues. With increasing age total body fat increases from 18% to 48% in females and from 18% to 36% in males. The amount of extracellular fluid remains unchanged but its proportion in the body increases with age along with a decrease in intracellular fluid which is a reflection of decreasing cell mass. All of these can have the effect of medications having a different volume of distribution than in younger subjects, since less or more of the medication may be retained in the circulatory or central compartment. For example for lipophilic drugs, volume of distribution (Vd) is increased.

Blood flow is reduced with age. Cardiac output declines roughly 1% yearly after the age of 25 and regional blood flow shows a similar yearly decline in flow to the brain (-0.35 to -0.5%), heart (-0.5%), liver (-0.3 to -1.5%), and kidneys (-1.1 to -1.9%). Corresponding changes in the ability of medications to distribute to less vascularized compartments such as fatty tissue and peripheral tissues will follow.

Plasma proteins remain roughly the same with age with the exception of plasma albumin in the frail elderly subject which can affect the Vd of highly bound acidic drugs with varying clinical significance; a greater free fraction of albumin bound drugs carries the potential for greater efficacy and toxicities. Alpha 1-acid glycoprotein (AAG) is increased in acute illness and chronic inflammatory diseases decreasing the free fraction of basic drugs such as propranolol (and tramadol) resulting in the potential for reduced efficacy in the elderly (39).

***Metabolism***

Hepatic metabolism or clearance of medications is related to the ability of the liver to biotransform medications to more easily eliminated metabolites and the ability of the cardiovascular system to present the drug to the liver where enzymes capable of metabolizing the drug are present. Hepatic clearance is the result of liver blood flow and hepatic extraction ratio. Depending on the ratio of hepatic clearance of a drug to the hepatic blood flow, extraction is generally classified as high (>0.7), intermediate (0.3-0.7) or low (<0.3) and represents the fraction of drug removed during one pass through the liver. Age related decrease in hepatic blood flow can reduce hepatic clearance of medications with a high hepatic extraction ratio and, in turn, increase bioavailability.

Several low extraction or capacity limited drugs metabolised by Phase I reactions have shown a significant reduction in clearance in the elderly. Cytochrome P 450 (CYP) enzymes are important in Phase I metabolic reactions of many medicines. CYP2D6, found in the liver and brain and CYP3A4 in the liver and gut, are important in the metabolism of opioid analgesics. CYP3A4 is responsible for metabolism of almost 50% of medicines as such medicines that utilise this metabolic pathway have a high potential for interaction with other medicines metabolised by CYP3A4, an important consideration in elderly patients who often take many medications. It has been shown in several studies that CYP2D6 remains unchanged with age while in some studies CYP3A4 has been shown to be reduced while others show no change (40-42). Studies have shown that monoamine oxidase activity is maintained with ageing (41, 43, 44). Conjugative metabolism is not generally affected by aging (45, 46).

***Excretion***

Renal clearance is reduced in the elderly and may have a variety of causes including reduced renal blood flow, reduced active tubular transport, loss of functional nephrons or all of these (37). Both glomerular filtration and maximum tubular secretion decline by approximately 0.6 percent per year after 25 years of age. Drugs having a high fraction excreted unchanged in urine will be mostly affected. However, it is difficult to distinguish the relative contribution of hepatic clearance and renal clearance to overall clearance, as both are susceptible to age related changes.

***Elimination***

Often half-life is prolonged in the elderly. In absence of intravenous drug administration in both young and elderly subjects, it is almost impossible to determine whether the net effect on half-life is related to alteration in total body distribution or clearance.

## 1.4 Pharmacology of pain

### 1.4.1 Pain treatment in the elderly

Pain is highly prevalent in the elderly, recent observational studies have shown that elderly patients are systematically undertreated (47-49). The selection of appropriate analgesics in elderly requires careful consideration of a variety of factors such as age related changes in body composition, co-morbid medical conditions and polypharmacy which can lead to heterogeneity in analgesic effect and side effects. The picture can be further complicated by the potential presence of frailty which is not necessarily tied to chronological age or impaired cognition.

A cross-sectional study of 21 380 nursing home residents aged 65 and older in nursing homes in 10 U.S. states found that the most common treatments for persistent pain were acetaminophen (37.2%), propoxyphene (18.2%), hydrocodone (6.8%) and tramadol (5.4%) (49). The 2008 consensus statement on opioid use for severe chronic pain in the elderly, focused their review on buprenorphine, fentanyl, hydromorphone, methadone, morphine and oxycodone (2). Thus opioids, including tramadol, are commonly used to treat a variety of

cancer related and non-cancer related pain conditions. Non-cancer related pain includes conditions such as low back pain, osteoarthritis and neuropathic pain.

Acetaminophen is a widely used analgesic and the drug of choice for mild to moderate pain on its own or in combination with stronger analgesics such as opioids (50, 51). It is frequently used to treat mild to moderate osteoarthritis and other painful conditions that affect the elderly. Although a recent meta-analysis of 137 studies comprising 33 243 participants found that acetaminophen was least likely amongst diclofenac, ibuprofen, naproxen, celecoxib, intra-articular (IA) corticosteroids and IA hyaluronic acid to be efficacious (52). There may be some effect of age on the PK of acetaminophen in healthy elders with reported results being variable (45, 53-55). Decreases in volume of distribution have been observed with increasing age (55). Frailty in older persons does seem to be associated with reduction in total clearance of acetaminophen in the elderly (45, 54-56), suggesting that in healthy older people intrinsic oxidative metabolism may be intact while in frail elderly it may be compromised (10). This is of particular concern with regard to unintentional overdose of acetaminophen, liver disease or use with alcohol amongst other factors putting the frail elderly patient at higher risk for formation N-acetyl-p-benzoquinoneimine and hepatic centrilobular necrosis (10). Recently, the safety of recommended doses of acetaminophen in the elderly are being questioned particularly in the frail elderly. Elderly people may have a worse benefit/risk ratio. Risk factors can include polymedication, glutathione depletion, organ insufficiency, malnutrition, dehydration and fragility (10, 57, 58).

Commonly used pain relievers such as ibuprofen and naproxen are non-selective cyclo-oxygenase inhibitors and carry a significant risk of cardiovascular events including death, gastrointestinal bleeding and kidney dysfunction and are used with extreme caution or not at all in the elderly. Oral nonselective and selective NSAIDs are rarely used in elderly patients due to the potential cardiovascular risks (10, 50-52). These side effects are generally not associated with use of tramadol and other opioids, making them an option for older patients with chronic pain (50).

When pain worsens, traditional opioids such as morphine, oxycodone, fentanyl, or buprenorphine may be added. There is debate about the value of tramadol and tapentadol, as these drugs having opioid and non-opioid mechanisms of action exhibit side effects and potential for drug interactions leading to serotonin syndrome. However, there is a general agreement that making use of multiple mechanisms of action (referred to as multimodal analgesia) along with non-pharmaceutical approaches provide better relief (51).

Treatment of moderate to severe pain in elderly patients is an important aspect of their care and opioids are considered an important tool (2, 50, 51, 59) with well-known efficacy but also potential for harm. Opioid related harm is significantly related to increasing age including risks of respiratory depression and falls and fractures (60). There are a variety of opioid options for treating elderly patients in pain, including morphine, codeine oxycodone, hydromorphone, fentanyl, tramadol, methadone, buprenorphine and tapentadol (59). Important considerations in the choice of an opioid in elderly especially frail elderly patients are the patient's renal function and the route of excretion of the opioid chosen, renally excreted opioids may accumulate in elderly people with impaired renal function (10, 59). Furthermore, since elderly patients are often taking many medications, understanding the potential for drug-drug interactions with opioids especially those metabolised by CYP enzymes such as tramadol and codeine is important. The choice of which opioid to use should be considered in the context of the characteristics of the individual patient, such as presence of complex comorbidities, psychosocial considerations, co-medications and careful management of side effects is a key consideration (59).

## 1.4.2 Opioid mechanism of action

Modulation of pain perception and response involves critical endogenous opioid systems and these systems are a major target of analgesic strategies (61, 62). Opioids are implicated in many molecular/cellular responses related to pain and affect including behaviours related to analgesia, reward, depression and anxiety. Opioid receptors are expressed in a variety of locations throughout the pain system including in afferent nociceptive neurons, the spinal cord and the descending modulatory pathways. In primary afferent nociceptive neurons, opioid receptors play an important role in the presence of

inflammation (63, 64). In the spinal cord and in pain modulating descending pathways, they directly inhibit interneurons, which in turn inhibit spinal cord transmission (62, 65). Descending pain control centres tend to have high concentrations of opioid receptors and endogenous opioids (66). There are four opioid receptor subtypes: μ, δ, k and opioid receptor-like 1 (ORL1) receptors. The μ receptor is the most ubiquitous opioid receptor in the spinal cord and is the main modulator of the pain system (67). In the spinal cord, 70% of opioid receptors are located pre-synaptically where they inhibit calcium influx by enhancing outward movement of potassium or inhibit adenylate cyclase conversion of adenosine triphosphate (ATP) to cyclic AMP (cAMP) and therefore, preventing the release of Substance P and CGRP (Figure 3). Post-synaptical opioid receptor activation results in inhibition of potassium ion efflux which, in turn, decreases neuron excitability. Opioids mainly excite the prefrontal cortex, hypothalamus, amygdala and cingulate gyrus resulting in an indirect excitation of neurons in the PAG, as well as also directly exciting PAG neurons projection to the RVM where they will affect ON and OFF cells by inhibiting opioid receptor bearing ON cells. They also inhibit GABAergic inputs to OFF cells leading to inhibition of the transmission of nociception (Figure 3).

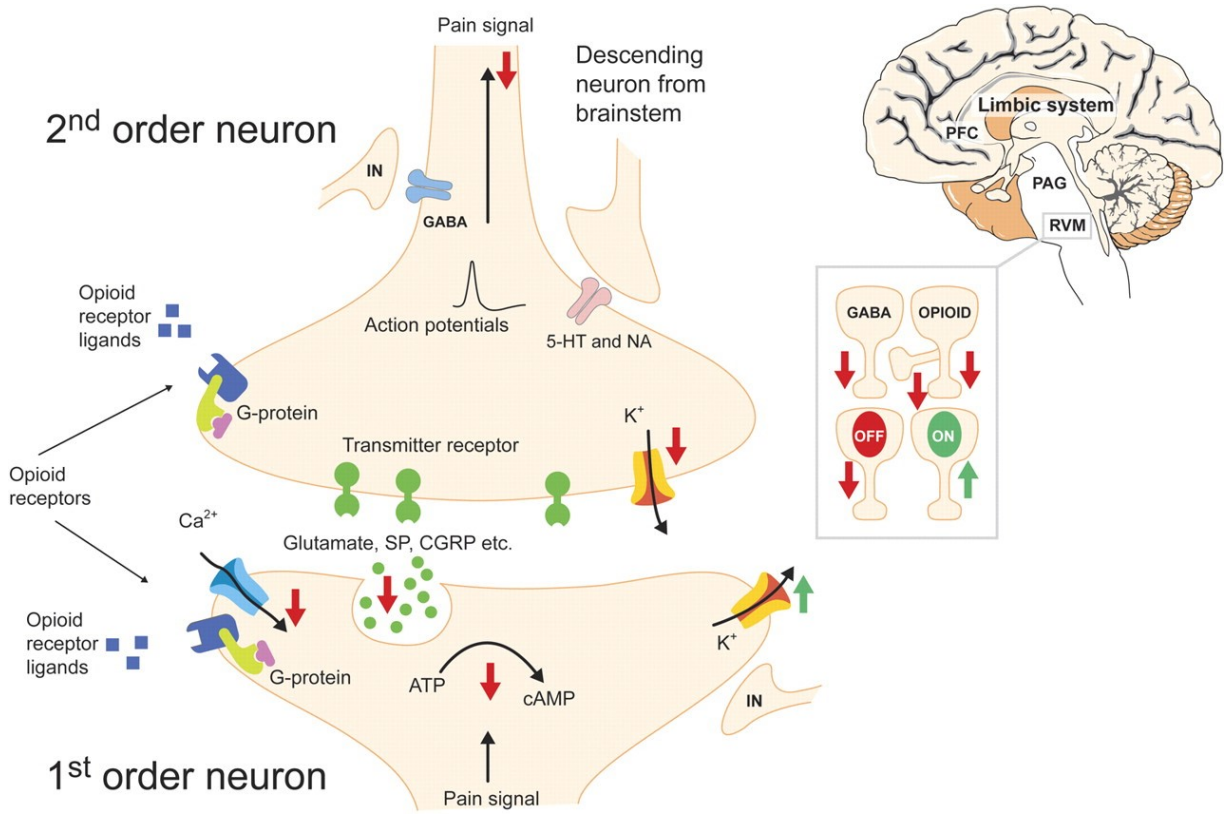


Figure 3. Opioid molecular mechanism of action in the spinal cord

5-HT – serotonin; ATP – adenosine triphosphate; $CA^{2+}$ – calcium ion; cAMP –cyclic adenosine monophosphate; CGRP – calcitonin gene related peptide; GABA – γ-hydroxy-butyric acid; $K^+$ - potassium ion; IN – interneuron; NA – noradrenaline; PFC – prefrontal cortex; PAG – periaqueductal grey matter; RVM – rostroventral medulla; SP – substance P (62). Reprinted from Olesen AE, Andresen T, Staahl C, Drewes AM. Human experimental pain models for assessing the therapeutic efficacy of analgesic drugs. Pharmacol Rev. 2012;64(3):722-79 with permission of Aspet journals.

Opioid receptors are 7-transmembrane spanning proteins that, following activation by an agonist, couple to inhibitory G-proteins (Gα and Gβγ subunits), then dissociate from one another and subsequently act on various intracellular effector pathways (61).

Opioids are currently the most efficacious analgesics for moderate to severe pain (68), yet their clinical utility continues to be limited by a compromise between efficacy and side effects, particularly in the elderly. The most common side effects of opioids can be divided into peripheral (constipation, urinary retention, hives, bronchospasm) and central effects (nausea, sedation, respiratory depression, hypotension, myosis, cough suppression) (61).

## 1.4.3 Tramadol

### 1.4.3.1 Tramadol mechanism of action

First synthesised in 1962, tramadol hydrochloride is a centrally acting analgesic which is structurally related to morphine and codeine (69). It is a racemic 1:1 mixture of (+)-tramadol and (-)-tramadol and is metabolised to (+/-)-*O*-desmethyltramadol (ODM; also known as M1) and (+/-)-*N*-desmethyltramadol as well as a number of other metabolites (70). The racemate, its enantiomers and the ODM metabolite, are all implicated in the production of anti-nociception through both non-opioid and opioid mechanisms (71). *In-vitro* and *in-vivo* studies have shown enantioselective pharmacology, pharmacokinetics and metabolism. These studies also reported that each enantiomer contributes to the analgesic effect of tramadol via different mechanisms of action and each enantiomer contributes synergistically to the drug effect (72, 73).

Tramadol acts as an opioid agonist by selectively binding to μ receptors in the spinal cord and brain (74, 75), although with much less affinity than codeine (1/10) and morphine (1/6000). The parent compound, tramadol, binds weakly to μ-opioid receptors; however, the (+)-ODM metabolite has 200 times the affinity of the parent drug. As a result, the opioid action of tramadol is thought to be primarily linked to the (+)-ODM metabolite (76, 77). Table 1 presents the affinities of tramadol, O-desmethyltramadol and their enantiomers as well as that of morphine for the μ-opioid receptor and serotonin (5-HT-2C) transporters.

Table 1. Relative affinity of racemic tramadol, tramadol enantiomers, O-desmethyltramadol and morphine

| | $K_i$ (μM) | | |
|---|---|---|---|
| | μ –opioid receptor | Serotonin (5-HT 2C) transporter | NE transporter |
| (+/-)-tramadol (78) | 2.4 | 0.78 | 0.90 |
| (+)-tramadol (78) | Not reported | 2.51 | 0.53 |
| (-)-tramadol (78) | Not reported | 0.43 | 2.35 |
| (+/-)-O-desmethyltramadol (79) | 0.0054 | Not reported | Not reported |
| (+)- O-desmethyltramadol (79) | 0.0034 | Not reported | Not reported |
| (+/-)-O-desmethyltramadol (79) | 0.24 | Not reported | Not reported |
| Morphine | 0.0012 | No effect | No effect |

The non-opioid mechanism of tramadol has been elucidated through studies that demonstrated a lack of reversibility of the analgesic effect by naloxone, lack of any naloxone induced withdrawal symptoms, production of mydriasis rather than miosis and reduction of analgesic effect with co-administration with non-opioid antagonists (71, 73, 80). In a study examining the actions of (+)-tramadol, (-)-tramadol and (+)-O-desmethyltramadol and (-)-O-desmethyltramadol on electrically evoked norepinephrine efflux and re-uptake in rat coeruleus brain slices, mean norepinephrine efflux was significantly ($p < 0.01$) increased by racemic tramadol (66%; SEM: 10%) and its (+)- enantiomer (57%; SEM: 10%) and (-)-enantiomer (64%; SEM: 13%). Norepinephrine re-uptake was blocked only by (-)-tramadol ($p < 0.01$), which increased the re-uptake half-time to 499% (SEM 63%) of pre-drug values. At the test drug concentrations, O-desmethyltramadol was inactive with regard to norepinephrine efflux or re-uptake (81). In a study of the actions of racemic tramadol, (+)-tramadol, (-)-tramadol and O-desmethyltramadol on electrically evoked serotonin efflux and uptake in rat dorsal raphe nuclei in the RVM, racemic tramadol and the (+)-tramadol enantiomer significantly blocked 5-hydroxytryptamine reuptake (both $p < 0.05$) and increased efflux (racemate: $p < 0.01$;

(+)-tramadol: $p < 0.05$) while O-desmethyltramadol and the (-)- enantiomer were inactive at the concentrations used in the study. (82) The non-opioid mechanism of action of tramadol involves activation of descending noradrenergic and serotonergic pathways (71). The (+)-tramadol enantiomer preferentially inhibits serotonin reuptake and enhances serotonin release while (-)-tramadol preferentially inhibits norepinephrine reuptake and enhances stimulation evoked norepinephrine release; (-)-ODM inhibits monoamine uptake (73, 83).

Several human studies using experimental pain models have demonstrated greater analgesic effect of tramadol compared to placebo (80, 84-86). Although designed to demonstrate efficacy versus placebo or other treatments, they do provide some information about onset and duration of analgesic effect which generally appears to occur within 2 hours of administration and to last until the end of the dosing interval (6 hours for immediate release (IR) formulations and 12 h for sustained release (SR) formulations this is limited by sampling frequency and lack of detailed presentation of onset and offset information (Table 1). In a study by Sarbu et al. (87), 47 patients with acute low back pain were administered a single 200 mg extended release tramadol tablet (intended for once daily administration). The patients indicated the time of onset of pain relief using the stopwatch method. Ratings of pain intensity and pain relief and pharmacokinetic samples were taken prior to dosing, at the onset of pain relief and 3 and 6 hours postdose. No rescue medication was permitted until the end of the study (6-hour postdose). Adverse events were monitored throughout the study. Onset of perceptible pain relief was achieved within 1 hour for the majority of patients and at plasma levels, suggesting a therapeutic threshold between 50 and 100 ng/mL.

Table 2. Summary of results from selected experimental pain models of tramadol

| | Desmeules et al. n =10[a] | Hummel et al. n = 20[b] | Hogger et al. n = 12[c] | Thurauf et al. n = 20[d] |
|---|---|---|---|---|
| Study design | Randomised, double-blind, placebo controlled, 4-way crossover | Randomised, double-blind, placebo controlled, 3-way crossover | Randomised, double-blind, 6-way crossover | Randomised, double-blind, controlled, 3-way crossover. |
| Experimental Pain Model | Transcutaneous electrical stimulation of the sural nerve | Tonic pain: dry airstream delivered to right nostril<br>Phasic pain: $CO_2$ stream applied to nasal mucosa | Electrical stimulation of the tooth pulp of central incisors | Phasic and tonic pain: $CO_2$ stream applied to nasal mucosa alternately in nasal cavity |
| Measurement | Electromyographic response measured on the ipsilateral biceps femoris<br>Pain threshold using a Pain Numerical Rating Scale | Chemo-sensory event related potentials (phasic stimuli)<br>Pain VAS | Somato-sensory Evoked potentials<br>8-point categorical pain intensity scale | Chemo-sensory event related potentials<br>Pain VAS |
| Treatment | Tramadol 100 mg, tramadol + yohimbine, tramadol + yohibine+naloxone, placebo | Tramadol IR 100 mg Tramadol SR 100 mg, Tramadol SR 150 mg, placebo | 50 mg tramadol, 50 mg tildine + 4 mg naloxone, bromofenac 25, 50 and 75 mg | Tramadol 100 mg SR, Tramadol 200 mg SR, placebo |
| Route of Administration | Oral | oral | Oral | oral |
| Onset of analgesia (h) | Not reported | <2 h | Not reported | < 2 h |
| Duration of Analgesia | 6h | 12h* | Not reported | > 12 h |
| Time to peak effect | 3.7 h | Not reported | Not reported | 6 h |
| Result | Both subjective (PNRS) and objective (nociceptive reflex/ RIII) pain threshold were increased | VAS and amplitudes of evoked potentials decreased, latencies of evoked potentials and EEG frequency spectrum unchanged | No parameters were affected | Decreased: VAS to tonic pain, amplitude of evoked potentials,, Unchanged: VAS to CO2 stimulation unchanged, latencies of evoked potentials unchanged |

[a] n=10 healthy young male volunteers mean age of 25.6 ± 4.5;[b] n = 20 healthy young volunteers (13 male and 7 female) mean age 27.8 years (range 23-41 years);[c] n = 12 healthy young volunteers (6 male and 6 female) mean age 25 ± 3.5 year; d n = 20 healthy young volunteers (10 male and 10 female) mean age 26.10 years (22-32 years) IR- immediate release typically administered q6h; SR- typically administered q 12h; VAS –visual analogue scale;

Studies of the analgesic effect of tramadol after use of a percutaneous electrical stimulation and cold pressor experimental pain models in CYP2D6 poor and extensive metabolisers have demonstrated greater analgesic effect among extensive metabolisers than among poor metabolisers, although poor metabolisers still achieved analgesia, possibly as a result of the non-opioid mechanisms of action of the parent compound (88, 89).

The efficacy of tramadol has been demonstrated in studies including elderly subjects up to 80 years of age in a variety of conditions including osteoarthritis, neuropathic pain, acute and chronic low back pain, post-operative pain and dental pain (90-97). It is indicated for moderate to severe pain at doses between 100-400 mg and requires titration to minimize side effects and achieve optimal efficacy.

### 1.4.3.2 Tramadol Pharmacokinetics

Tramadol and ODM have been shown in humans to have stereoselective pharmacokinetics and metabolism (98). Steady state concentrations of (+)-tramadol were found to be approximately 30% higher than (-)-tramadol and (+)-tramadol half-life was approximately 1 hour slower. Serum concentrations of (-)-ODM were found to also be approximately 30% higher than (+)-ODM in 12 healthy young (18-22 years of age) male subjects administered as a single oral 100 mg dose of tramadol sustained release tablets twice daily for 11 days. Of note the volunteers were not screened for CYP2D6 status. It is not expected that these differences are clinically significant (98). A population pharmacokinetic (popPK) analysis of two studies: one in 12 healthy young male volunteers and a second in 24 healthy young (22-26 years of age) volunteers (12 males and 12 females) administered intravenous (I.V.) and oral tramadol found similar results and furthermore that the enantioselectivity appears to be administration route dependent (99).

After I.V. administration, tramadol has an initial distribution phase with half-life of 6 minutes, which consists of a faster distribution into tissues of the central compartment consisting of blood and highly perfused tissues (e.g., kidney, liver) and a slower distribution phase with a half-life of 1.7 hours for equilibrium between tissues of the peripheral compartment and the blood (72, 100) A bioavailability study comparing 10 healthy young male subjects administered intravenous and oral tramadol as a 100 mg single dose found a

volume of distribution of 203 L after I.V. administration, of 306 L after oral administration indicating high tissues affinity (Table 2). The authors also found absolute bioavailability was 68% ± 13% mostly due to hepatic first pass effect (100). Tramadol is approximately 20% plasma protein bound.

After oral administration of immediate release capsules, tramadol is rapidly (30 minutes) and completely absorbed. It takes 1.9 hours to attain peak plasma concentrations of 409 ng/mL following single dose administration of 100 mg (Table 1). Both maximum plasma concentrations ($C_{max}$) and area under the time-concentration curve (AUC) demonstrate a linear increase over the dose range of 50 to 400 mg. Oral administration of 100 mg four times a day over seven days results in a 16% higher $C_{max}$ and 36% higher AUC compared to single dose oral administration of 100 mg, thus oral bioavailability increases to 90-100% possibly as a result of saturated first-pass metabolism (72). Volunteers fed a high fat breakfast had a 17% higher $C_{max}$ and a 10% higher AUC than fasted volunteers. This difference was not regarded as clinically significant (101).

Tramadol is 90% renally excreted, the remainder of a radioactively labelled dose was recovered in feces (102). Following oral administration, the mean apparent total clearance was 45 l/h and the mean elimination half-life approximately 5 hours (100).

Table 3. Summary of pharmacokinetics of a single 100 mg oral dose of tramadol in healthy young and elderly subjects

| | Lintz et al. (100) | | Lee et al. (72) | | Karhu et al. (103) |
|---|---|---|---|---|---|
| Pharmacokinetic parameter | IV Mean ± SD (CV%) (n = 10) | Oral Mean ± SD (CV%) (n = 10) | 65-75 years (n = 12) | ≥ 75 years n = 18 | TOAD young 24 hrs (n=26) |
| $k_a$ ($h^{-1}$) | 3.5 ± 3.2 (91%) | 2.15 ± 0.93 (43) | NP | NP | NP |
| $C_{max}$ (ng/mL) | 409 | 290 ± 57 (20) | 324 ± NP (NP) | 415 ± NP (NP) | 91 ± 27 (30) |
| $t_{max}$ (h) | NA | 1.90 ± 0.50 (26) | 2.0 ± NP (NP) | 2.1 ± NP (NP) | 9[c] (3-16) |
| $k_{el}$ ($h^{-1}$) | 0.137 | NP | NP | NP | 0.106 ± 0.026 (25) |
| $t_{1/2}$ (h) | 5.16 ± 0.81 (16) | 5.13 ± 0.81 (16) | 6.1 ± NP (NP) | 7.0 ± NP (NP) | 6.11 ± 1.31 (21) |
| Vd/F (L) | 203 ± 40[a] (20) | 306 ± 52[b] (17) | NP | NP | 502[d] |
| $Cl_{tot}$/F (L/h) | 28± 7.44 (27) | 43 ± 10 (25) | 47.8 ± NP NP | 29.5 ± NP NP | NP |
| AUC (ng*h/mL) | 3802 ± 994 (26) | 2513 ± 770 (31) | 2508 ± NP NP | 3854 ± NP NP | 2108 ± 731 (35) |

[a] $Vd_\beta$ – volume of distribution ; [b] $Vd_\beta$/F – apparent volume of distribution; [c] Median and range
[d] $Vd_\beta$/F calculated from data provided Dose/AUC*$k_{el}$

AUC – area under the plasma concentration time curve; $C_{max}$ – maximum plasma concentration; $Cl_{tot}$- apparent total body clearance; h – hours; I.V.– intravenous; $k_a$ – constant rate of absorption; $k_{el}$ – constant rate of elimination; NA – Not applicable; NP- Not provided; SD – standard deviation; $t_{½}$ - half-life; $t_{max}$ – time to maximum concentration; TOAD – tramadol once daily dosing; Vd – apparent volume of distribution

Tramadol is metabolised in the liver. Animal studies have identified 11 metabolites in the urine, 5 from phase I reactions and 6 from phase II reactions. CYP2D6 enzymes are responsible for the formation of (+/-)-*O*-desmethyltramadol (ODM or M1) while CYP3A4 and CYP2B6 enzymes are implicated in N-desmethyltramadol (NDM or M3) formation (102). Both (+/-)-*O*-desmethyltramadol (ODM) and (+/-)-*N*-desmethyltramadol undergo additional phase I metabolism and the resulting demethylated compounds are further conjugated. Tramadol, ODM and NDM excretion in 24 hour urine was determined to be 12%, 15% and 4% of the administered dose (70). Since the (+)-ODM metabolite is the main activator of the opioid mechanism of action of tramadol, CYP2D6 plays an important role in analgesic response with tramadol. CYP polymorphisms may be the source of variability in individual PK and PD parameters with tramadol (70, 101).

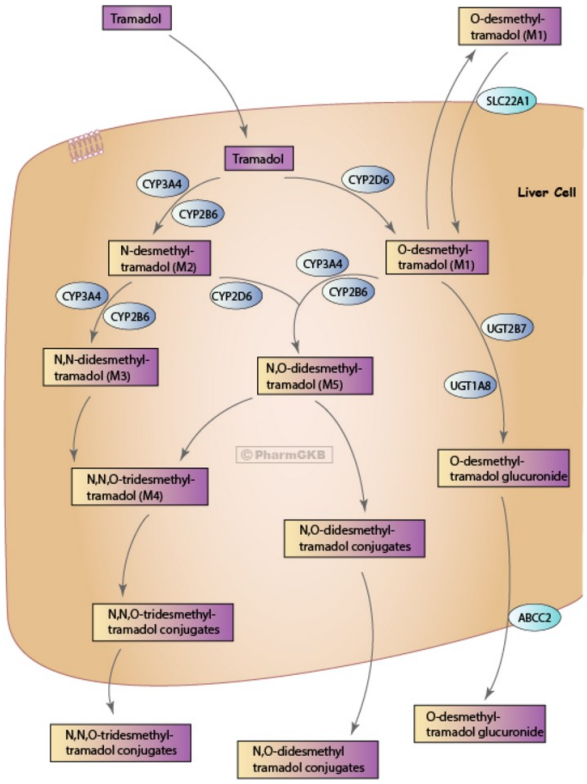


Figure 4. Tramadol metabolism in the human liver cell

CYP – Cytochrome P450.

Reproduced with permission of Wolters Kluwer Health, Inc. Gong L, Stamer UM, Tzvetkov MV, Altman RB, Klein TE. PharmGKB summary: tramadol pathway. Pharmacogenet Genomics. 2014;24(7):374-80 (104). Tramadol Pharmacokinetics https://www.pharmgkb.org/pathway/PA165946349#tabview=tab0&subtab=

Given the importance of hepatic function in the metabolism of tramadol into the more potent ODM metabolite and the fact that tramadol and its active metabolite are primarily excreted by the kidney, age related changes in hepatic and renal function may affect the PK and PD of tramadol.

Lee et al. (1993) report from data on file of the manufacturer regarding tramadol PK in elderly subjects as well as those with renal impairment and hepatic insufficiency (72). In comparing I.V. and oral administration in young healthy volunteers with oral administration in 12 subjects 65-74 years of age and 10 who are 75 or older (Table 2), they conclude that there was a trend for absolute bioavailability to increase with increasing age and terminal half-life

was also prolonged, although neither reached statistical significance. The authors indicate that the increased bioavailability can be explained by an age-related decrease in hepatic dysfunction, although they do not mention whether and what degree of hepatic dysfunction the subjects studied had. The elimination half-life of tramadol was found to be 1.5 to 2 times longer in patients with mild renal impairment (creatinine clearance 50-80 mL/min). In patients with liver cirrhosis plasma concentrations and elimination half-life were found to be increased by a factor of 2-3 compared to young healthy volunteers. Prolongation of dosage interval was recommended both in patients with renal impairment and hepatic impairment although not in the elderly (72). In the research presented here, we used a once-daily formulation of tramadol which has been demonstrated to be bioequivalent to immediate-release and twice-daily controlled-release formulations with respect to exposure (based on AUC) (Table 3) (103). The formulation used is composed of an outer-compression coat which contains 25% of the total daily dose and which releases immediately. The core of the tablet contains the remainder of the daily dose which is released in a controlled fashion over 24 hours (105).

Table 4. Pharmacokinetic parameters tramadol and ODM in healthy young subjects after oral administration of immediate release tramadol and extended release tramadol

| Parameter (n=24) | Tramadol OAD 200 mg single dose | Tramadol IR (50 mg every 6 h for 4 doses) | Point Estimate for the difference, %, [90% CI] |
|---|---|---|---|
| Tramadol | | | |
| $AUC_{0-\infty}$ (ng*h/mL) | 5582 ± 2535 | 5851 ± 2456 | 92.8 [87.2-98.7] |
| $C_{max}$ (ng/mL) | 256 ± 90 | 353 ± 118 | 71.6 (66.9-76.7) |
| $t_{1/2}$ (h) | 7.07 ± 1.42 | 6.03 ± 1.31 | 117.6 [111.8-123.7] |
| $\lambda_z$ ($h^{-1}$) | 0.102 ± 0.020 | 0.120 ± 0.027 | - |
| $t_{max}$ (h) | 6.0 (2.5-16.0) | 16.0 (6.5-20.0) | $p < 0.001$ |
| ODM | | | |
| $AUC_{0-\infty}$ (ng*h/mL) | 1430 ± 497 | 1429 ± 407 | 98.1[92.4-104.0] |
| $C_{max}$ (ng/mL) | 96.6 ± 18.8 | 70.2 ± 19.7 | 79.8 [75.9-83.9] |
| $t_{1/2}$ (h) | 7.81 ± 1.60 | 6.86 ± 1.52 | 114.2 [107.5-121.4] |
| $\lambda_z$ ($h^{-1}$) | 0.092 ± 0.019 | 0.106 ± 0.024 | - |
| $t_{max}$ (h) | 9.0 (4.0-16.0) | 19.5 (13.0-22.0) | $p < 0.001$ |

$AUC_{0-\infty}$ – area under the concentration time curve from time 0 to infinity; $C_{max}$ – maximum plasma concentration; h – hours; $\lambda_z$ – rate constant of elimination; SD – standard deviation; $t_{½}$ - half-life; $t_{max}$ – time to maximum concentration

## 1.5 Experimental pain models

Few new analgesics have been approved in recent years, despite promising results in animal models (62). The complexity of the pain response in humans, including cognitive, behavioural and emotional aspects of pain, may limit the translation of animal results to humans. At the same time, the multiple confounding factors in studies of patients, such as disease state, concomitant medications and emotional state of the patient make it difficult to

evaluate analgesic effects and specific mechanisms in patients with pain, resulting in frequently inconclusive studies. Therefore, experimental pain models in humans may provide useful additional information in the development of analgesics.

Careful design of studies that can mitigate confounding factors should take into consideration 3 main aspects (106, 107):

- PK and PD of the medicine: This should include an understanding of the onset and duration of analgesia, maximum effect, any dose limiting side effects and amongst other consideration any special metabolic features such as CYP polymorphisms
- Suitability of the pain model: This should take into account the pain pathways and mechanisms involved by the experimental pain stimulus but also which pain pathways and mechanisms are affected by the medicine.
- Opportunities to optimise assay sensitivity: These aspects include the number and frequency of observations, dosing interval of the medication and its relationship to the former, determination of and appropriate control (active and/or placebo), and study design factors for ensuring control such as blinding and randomization.

Placebo control is traditionally accepted by the scientific community as the best way to determine the true effect of a medication, based on the premise that there is an underlying effect of placebo and that true medication effect is additive to that of the placebo effect (108, 109). That being said, placebo response is highly variable and depends on many contextual factors (110), this is particularly true in analgesic studies. Vase et al., in their meta-analysis of 21 articles published between 2002 and 2007, found a highly variable magnitude of placebo analgesia with effect size calculated using Cohen's D ranging from 0.12 to 2.51. (110-113). This highlights the importance of carefully choosing the experimental pain model and optimising assay sensitivity by utilising, where possible a double blind, randomised trial design.

Standardised neuroselective nerve conduction threshold tests can be used to objectively evaluate the integrity of sensory nerves from the periphery to the central nervous system or the effect of analgesic drugs, including opioids, on nociception (114-118). Vibratory sensations can be used to evaluate large fibre sensory transmission or temperature, heat or cold can be used to evaluate sensory transmission of smaller diameter sensory fibres (Aδ and C fibres). Both can be confounded by variations in skin thickness and temperature and vibratory tests can be affected by variations in bone conductance. Transcutaneous electrical stimulation has been demonstrated to excite Aδ and both polymodal and stimulus specific C-fibres. Electrical stimuli directly stimulate nerve fibres, bypassing free nerve endings and therefore are not affected by skin thickness(119).

Data on opioid sensitivity based on either thermal or current sensory stimulation are conflicting. A study examining the effect of remifentanil (an opioid analgesic) on heat pain tolerance threshold showed no difference from saline in healthy volunteers (117). Conversely, a comparative study of different experimental pain models used to establish alfentanil efficacy, found that electrical stimuli were sensitive enough to assess the concentration-response relationship (120). Gustorff et al. (116) found that while both heat and current sensory testing were able to detect an analgesic effect with remifentanil, current sensory testing was more responsive than heat sensory testing. Furthermore, Tucker (31) indicates that electrical stimuli have a safety advantage over heat/cold stimuli, in that at threshold, they do not induce any injury or lesion.

Electrical current stimulation predominantly stimulates C, Aδ and Aβ fibres (116). Low threshold afferents such as Aβ fibres are myelinated fibres involved in innocuous sensations such as light touch, vibration, and pressure. Aδ fibres and C fibres are high threshold afferents which convey pain and temperature sensations (121). The device selected to provide the electrical stimulation for the ESPM in this study is the Neurometer® CPT/C. This device utilizes the fact that large diameter sensory neuron fibres, such as Aβ fibres can respond to high frequency rapid stimulus (2000 Hz) while small fibres such as C and Aδ fibres require several milliseconds of continuous depolarization with low frequency stimulus (e.g. 5 and 250 Hz, respectively). The neurometer uses these 3 frequencies of electrical sinewave

stimulus to neuroselectively stimulate the 3 types of neuron fibres (122). In our research we studied Aδ fibres and C fibres which convey pain sensations.

The Neurometer® CPT/C is a fully automated quantitative neuro-diagnostic device and methodology typically used to evaluate CPT to evaluate sensory pathologies. The Neurometer® can also be used to measure PTT, the maximum amount of the electrical stimulus that is atraumatic and that a volunteer is willing to tolerate.

The electrical stimuli were administered based upon the standardized testing methodologies for obtaining Current Perception Threshold (CPT) and Pain Tolerance Threshold (PTT) data (123, 124).

The Neurometer® device generates constant alternating current sinusoid waveform stimuli at 3 different calibrated frequencies. The possible stimuli range from 0.01 milliAmperes (mA) to 10 mA for each frequency. Each of the three frequencies evokes a different sensation. At least one minute rest must be allowed between application of each of the frequencies and the frequencies should be administered in descending order (2000 Hz, then 250 Hz then 5 Hz) to avoid desensitization of the sensory fibres

A pair of 1 cm diameter gold electrodes separated by a mylar strip is used to deliver the current to the skin surface (125, 126) (Figure 5). Gold plated electrodes are used to ensure the best conductance as standard silver or carbon electrodes distort the sine wave test stimulus. The skin is cleaned with a prep paste to reduce any excess tissue resistance or impedance that can effect the delivery of the test stimulus (126). An electrode gel is applied to the surface of the electrodes before placement at the site to be tested to ensure good contact with the skin and good conductivity. The electrodes are held in place by a non-conductive strip of tape.

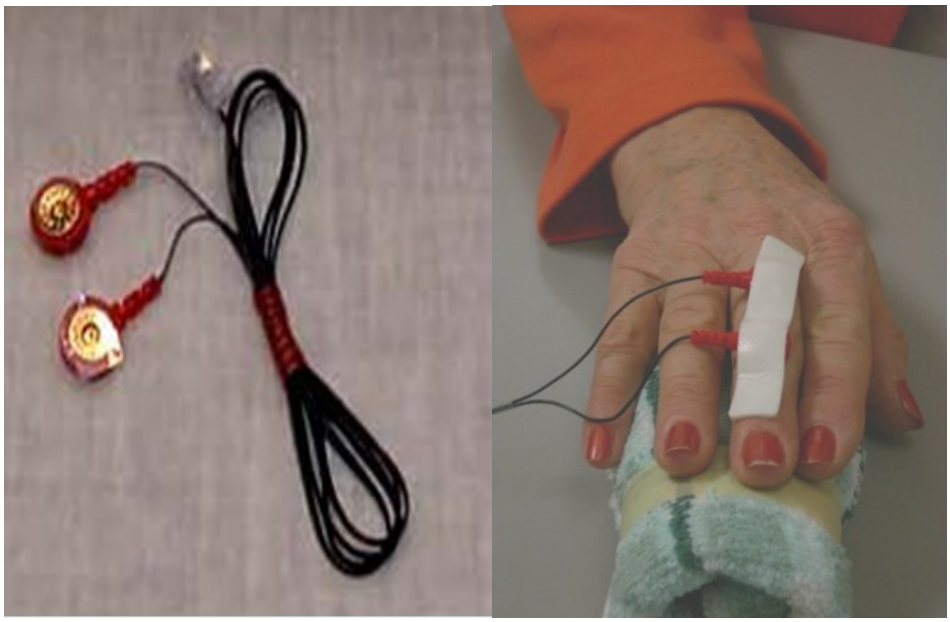

Figure 5. Electrodes and placement of electrodes for use with the Neurometer®

A computer attached to the Neurometer® is used to select the frequency of the stimulus and initiate and stop the painful stimulus and record the pain tolerance of the subject in μ amperes (μA) (Figure 6).

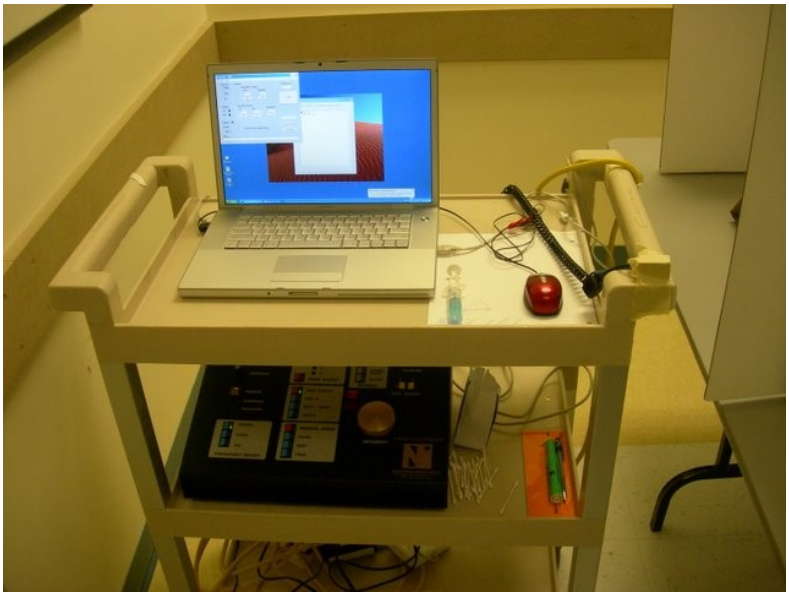

Figure 6. Neurometer® device and computer control

During PTT testing the Neurometer® increases the threshold intensity in a stepwise fashion with the 250 Hz frequency increasing in 25 μA increments with 1.5 second (s) on/off cycle and the 5 Hz increasing in 12 μA increments with a 1.7 s on/off cycle.

The Neurometer® has been used in both experimental and diagnostic settings. In 2001, Angst et al. (127) studied the analgesic effects of sustained release hydromorphone as compared to immediate release hydromorphone and placebo in young subjects (mean age 27 years). They were able to use PPT and PTT data collected by the Neurometer® to demonstrate that peak analgesia occurred significantly later (9 h versus 1.5 h for the sustained release and immediate release formulations, respectively) and that analgesia lasted significantly longer with the sustained release formulation. Gustorff et al. (116) evaluated PPT and PTT to both heat, cold and electrical stimulation at 5 Hz, 250 Hz and 2000 Hz in remifentanil versus placebo. For remifentanil, they were able to detect an analgesic effect for pain induced by current at 5 Hz, 250 Hz but not at 2000 Hz. This is not surprising since 2000 Hz stimulates Aβ fibres which detect sensations such as light touch, vibration, and pressure. They were also able to detect a significant analgesic effect in the remifentanil group to heat induced pain but not for cold induced pain.. In another study, Gustorff (128) also used the Neurometer® to assess pain threshold for electrical stimulus at 5 Hz and 250 Hz and demonstrated a lack of acute tolerance during remifentanil infusion in healthy volunteers. Skarke et al. (118) used the Neurometer® to examine the effects of I.V. morphine and morphine-6-glucuronide and placebo using the 5 Hz stimulus. They found comparable effects on pain tolerance at 5 Hz between morphine and morphine-6-glucuronide but that there was a longer delay in the time course of effects for morphine-6-glucuronide. Furthemore, these effects were only achieved at high amounts of systemic morphine-6-glucuronide suggesting that it barely contributes to CNS opioid effects after administration of analgesic doses of morphine. Thus, the Neurometer® can be used to effectively assess PTT and PPT in experimental pain settings.

# Chapter 2 : Pharmacometric modelling of analgesics

## 2.1 Importance of pharmacometrics in special populations

Academic and pharmaceutical industry researchers and human medicines regulatory agencies are increasingly recognizing the importance of PK modelling in the development of human medicines. PK is a particularly important tool in understanding the PK and PD of medicines in small and special populations such as orphan and rare orphan disease populations, pediatrics and the elderly populations and populations with renal and/or hepatic insufficiency.

The European Medicines Agency (EMA) identifies the concept of extrapolation and defines it as (129):

> *Extending information and conclusions available from studies in one or more subgroups of the patient population (source population(s)), or in related conditions or with related medicinal products, to make inferences for another subgroup of the population (target population), or condition or product, thus reducing the need to generate additional information (types of studies, design modifications, number of patients required) to reach conclusions for the target population, or condition or medicinal product.*

Indeed quantitative numerical and modelling techniques have been used to facilitate the integration of pre-clinical and clinical development data and provide a rational basis for dosage regimen design and optimization of treatment (130). The EMA go on to indicate the PK/PD modeling is an important tool for the process of extrapolation which has its goal as avoiding unnecessary studies and optimising decision-making when patients are scarce, although the discussion paper refers to pediatric patients it makes the point that the principles and processes proposed are relevant in any case where patient numbers are limited or the conduct of studies could cause ethical issues or be practically impossible. This is of relevance in older populations, particularly in the frail elderly. Pharmacometrics can be instrumental in

giving regulatory agencies a scientific basis to grant access for these patients who often have high unmet medical need and few, or no treatment options.

## 2.2 Pharmacometrics

Pharmacometrics can be described as (131):

> *"the science of developing and applying mathematical and statistical methods to (a) characterize, understand, and predict a drug's PK and PD behavior; (b) quantify uncertainty of information about that behavior; and (c) rationalize data-driven decision making in the drug development process and pharmacotherapy"*

Noncompartmental (NCA) and compartmental approaches are important types of models used by pharmacometricians and formed the basis for the models used in the research presented herein. It has been said that while noncompartmental approaches describe data, population approaches describe systems (Figure 5) (132). Either can be based on modelling individual data and compartmental analyses can also utilize population methodologies.

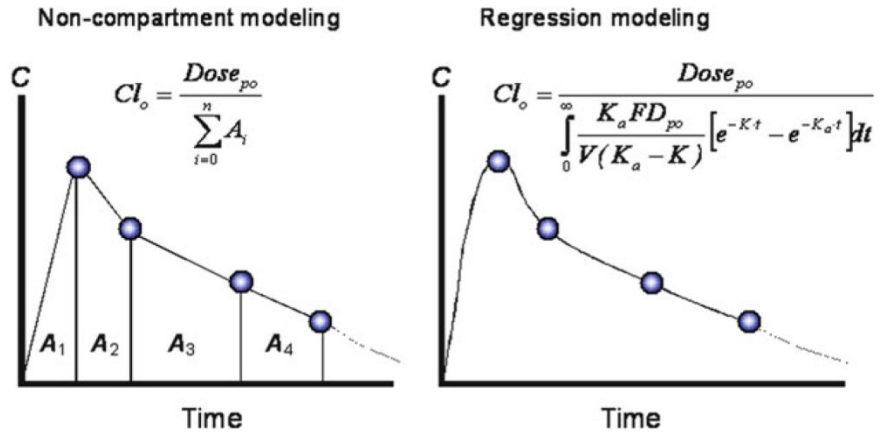


Figure 7. Comparison of NCA (left) and nonlinear regression modelling (right).

Ka, K and V in the right-hand panel indicate the model parameters to be estimated by regressing the model to data.

Gabrielsson J, Weiner D. Non-compartmental analysis. Methods Mol Biol. 2012;929:377-89. (132) with permission of Springer.

## 2.2.1 Noncompartmental analysis

### 2.2.1.1 Pharmacokinetics

A NCA is a mathematical description of data that utilises statistical moment analysis to determine the degree of exposure to a medicine (area under the curve (AUC)) and estimate PK parameters such as clearance, elimination half-life ($t_{½}$), $C_{max}$ and time to maximum concentration ($T_{max}$) (132). NCA is primarily descriptive and analyses each subject separately, without the possibility to carry out simulation (133). It has several advantages including that it requires very few assumptions, only that input and output occur from the central compartment and that the PK of the medicine must be linear. NCA can be conducted reasonably rapidly and if a study is well designed, with adequate sampling, it can provide a robust description of the data but little information is provided about the system from which the data is derived (133, 134).

The area under the curve for a NCA is calculated using the linear or log-linear trapezoidal rule. This is accomplished by calculating the area of each of the trapezoids that make up the plasma-concentration versus time curve (eq. 1) and then integrating them (eq. 2).

$$\Delta \text{ AUC} = \frac{(C(t_1) + C(t_2)) \cdot (t_2 - t_1)}{2} \qquad \text{(eq. 1)}$$

Where Δ in AUC represents the change in the trapezoid over the estimation interval, $Ct_1$ is the plasma concentration at the beginning of the interval of the trapezoid and $Ct_2$ is the plasma concentration at the end of the interval of the trapezoid; $t_2$ is the time at the end of the interval and $t_1$ is the time at the beginning of the interval.

The integral equation for the zero$^{th}$ moment is (eq. 2):

$$\text{AUC} = \int_0^\infty C(t)dt \qquad \text{(eq. 2)}$$

And area under the curve for the first moment (AUMC) is (eq. 3):

$$\text{AUMC} = \int_0^\infty t \cdot C \cdot dt \qquad \text{(eq. 3)}$$

Where *t* is time, C is plasma concentration and d*t* is the difference in time, for the trapezoidal intervals defined between 0 and infinity (∞). AUMC has no physiological value, rather it is a mathematical variable used to calculate other PK parameters that are more meaningful physiologically, such as mean residence time (MRT).

MRT is calculated as per (eq.4) and the apparent volume of distribution at steady state ($Vd_{ss}$) as per (eq.5). MRT reflects the average time that a drug molecule spends in the body and can be used to help interpret the duration of effect for directly acting molecules. For an

orally administered medicine, it must take into account mean input time (MIT). $Vd_{ss}$ reflects the volume of distribution at steady state and is independent of elimination, the free concentration in plasma is equal to the free concentration in the body. It is unique in that it provides the overall elimination rate constant for a multicompartment model. A single compartment model has a single overall rate constant so this is less relevant.

$$MRT_{oral} = \frac{AUMC}{AUC} - MIT \qquad \text{(eq. 4)}$$

$$Vd_{ss} = D \cdot \frac{AUMC}{AUC^2} \qquad \text{(eq. 5)}$$

Sampling schedule plays an important role in the magnitude of error associated with the estimated width of each trapezoid. In general, it should be less that the expected distribution or elimination half-life, depending on the sampling period. Since NCA assumes that absorption or elimination rate is first order, this can result in overestimation of the ascending or absorption phase and underestimation of the descending or elimination phase. The sampling schedule can emphasise this issue if the time between sampling is large relative to the $t_{½}$ . This concern is also relevant to the calculation of MRT and $Vd_{ss}$. This is addressed by calculating the AUC from time zero to infinity also known as the extrapolated area (eq. 4)

$$AUC_{t_{last}}^{\infty} = \int_{t_{last}}^{\infty} C_{last} \cdot e^{-\lambda z\,(t-tlast)}\, dt \qquad \text{(eq. 4)}$$

Where $C_{last}$ and $\lambda_z$ are, respectively, the last measurable non-zero concentration and the terminal slope on a log scale. The terminal slope is obtained from the terminal slope of the semilogarithmic concentration time curve, which for accurate measurement must have at least 3 observations. Usually, we expect that the extrapolated area is less than 20% of the overall AUC for an adequate characterization of the PK profile.

### 2.2.1.2 Pharmacodynamics

Non-compartmental analyses have also been found useful to characterise the PD response to a given dose of medication (135-138). It is based on the integration over time of the effect expressed either as an amplitude or as a surface area under time intervals. The sum of pain intensity differences (SPID), an approach similar to the AUEC, but which does not use time to weight the pain intensity scores, has been used in analgesic clinical trials to assess treatment efficacy (139, 140). The time weighted SPID score was subsequently proposed in 1982 (136). Trials of analgesic response often make endpoint analysis the method of choice for analysis. However, for assessment of repeated measures after single dose analgesia, an AUEC approach can take into account the response, which may vary considerably over the time period where analgesic effect is expected to increase and wane. Thus AUEC approaches have the advantage of taking into account the time course of response and the order in which data were obtained (138).

Response data can be complicated by the presence of a non-zero baseline effect and in the case of analgesics and most medications, a placebo effect. The total analgesic effect will be composed of the true effect of the medication and the placebo effect. Both of these can vary over the time course of the analysis; response may not return to baseline at the end of the dosing interval and placebo response is normally high early in administration before declining. However, it may wax and wane throughout dosing based on a variety of individual and environmental factors (136, 141). Scheff et al. (138) suggest accounting for biphasic responses, i.e. both positive and negative changes in values. Figure 6 illustrates this concept.

Generally there are 3 approaches to establishing baseline:

**Estimation from time zero ($t_0$) values only:**

This approach is useful when no separate control is available and baseline measures are only taken prior to administration. Baseline, in this case, is computed by assuming that baseline response stays constant at the original value throughout the administration period and makes sense in data that do not generally return to baseline.

**Estimation from $t_0$ and last evaluation:**

This approach is useful where baseline values exhibit a perturbation and then return to baseline such as with acute dosing when enough time is given to ensure that washout occurs. The first and last time points are used to estimate the true baseline.

**Estimation from a separate control group or condition (placebo administration)**

The third case can only be applied when measurements for a separate control group or condition are available at each time point. Baseline varies with time and conditions (e.g. circadian rhythm) and the ratio of the treated and untreated control condition can be compared and used as part of a model.

As with AUC analysis, AUEC makes use of the linear or log-linear trapezoidal rule by calculating the area of each of the trapezoids that make up the effect (E) versus time curve and then integrating them.

$$\Delta \text{ AUC} = \frac{(E(t_1) + E(t_2)) \cdot (t_2 - t_1)}{2} \qquad \text{(eq. 5)}$$

$$\text{AUC} = \int_0^{\infty} E(t)dt \qquad \text{(eq. 6)}$$

For the calculation of AUEC, both positive and negative fluctuations from the predetermined baseline response can be calculated as well as summation of all positive and negative partial AUEC which yields a net AUEC (NCA Model 220, Pharsight Corp., Mountain View, CA, USA).

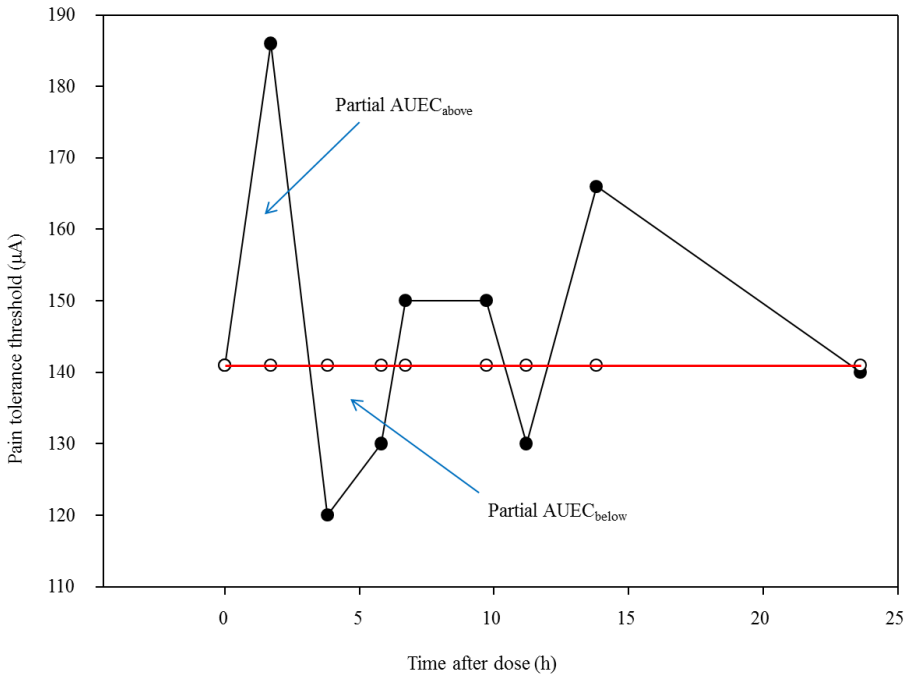


Figure 8. Illustration of positive and negative AUEC versus baseline.
Red line indicates the baseline PTT, black line indicates the response over time from which, using the linear trapezoidal rule, AUEC is calculated.

## 2.2.2 Population modelling

Population methodologies evaluate the data from all the individuals in a population at once, usually using a nonlinear mixed effects model (142). Developed in the 1970's and through the work of Sheiner and Beal the discipline has grown in sophistication and prominence with the evolution of computing power. Sheiner and Beal (143) sought an approach to modeling pharmacokinetic data that was between the individual (naïve pooled) approaches which fitted all of an individuals' data together as though there were no

differences in individuals' PK and the two-stage approach which fitted each individual's data separately and then took the means of the individual parameters. They found that applying these approaches to the same data resulted in differences in the population parameter estimates. Using simulations, they investigated these difference and found that using the pooled data approach failed to estimate variabilities and produced imprecise estimates of mean kinetics while the two-stage approach produced good estimates of mean kinetics, but biased and imprecise estimates of inter-individual variability. So they proposed a third approach, using nonlinear mixed effect modeling which produced accurate and precise estimates of all parameters, and also reasonable confidence intervals for them (143-146). Population models permit the simultaneous analysis of sparse or rich data or highly heterogeneous data, including data from healthy volunteers and patients or even across studies and across a variety of age groups (147, 148).

The basic equation to describe concentration or effect (Y) in a given individual (*i*) at a given time (*j*) is:

$$Y_{ij} = f(X_{ij}, \theta + \eta_{ij}) + \varepsilon_{ij} \qquad \text{(eq. 7)}$$

Where *f* represents the non-linear function of a compartmental model, $X_{ij}$ represents the fixed effects (e.g. dose, time, covariance matrix), $\theta$ represents the vector of typical values in the population, $\eta_i$ represents the vector of random effects that quantifies the deviation between the population and the individual random effect and $\varepsilon_{ij}$ represents deviation of the residual variability between the predicted value from the model and the observed value in a given individual. Generally we assume that $\eta_{ij}$ follows a normal distribution centred around zero with a variance of $\omega^2$ ($\eta_i \sim N(0, \omega^2)$, while $\varepsilon_{ij}$ follows a normal distribution centred on zero with a variance of $\sigma^2$) ($\varepsilon_{ij} \sim N(0, \sigma^2)$.

Population modeling permits the identification and description of relationships between a subject's physiologic characteristics and observed drug exposure or response (147).

As such, a sound understanding of the influence of factors such as weight, age, genotype, renal and hepatic function and concomitant medications on drug exposure and response is critical to developing models that are a sound basis for dosage recommendations and understanding the effect of dosage on the efficacy and safety of medicines (147). Another important aspect of a well-articulated model is that it is fit for purpose, many different models can be used to describe a system depending on the purpose of the modelling exercise, what is important is that the objective of the modelling exercise is well understood and that the model has credibility and fidelity; these are two key aspects of being fit for purpose. In credible models assumptions are clearly understood and delineated; the model conforms to accepted principles and mechanisms and can be justified and defended. Models with fidelity retain key components of the real systems or processes they represent (147). Finally, the principle of parsimony is an important aspect of population modeling; we seek always to find the simplest model that best describes the PK and/or PD effect and the variability within the population. The objective of population modelling is to identify a mathematical function that describes the time-course of concentration and/or effect of a medicine and the different levels and sources of variability within the population.

The development of a population PK (popPK) model involves:

- a structural model which describes the system in terms of virtual compartments which usually correspond to organs or tissues or groups of organs or tissues;
- a statistical model that quantifies, describes and explains the intra-individual, random and other sources of variability seen in the population.

To accomplish this, population modelling makes use of the maximum likelihood:

$$L_i ( Y_i \mid xi, \theta, C_i = \sum_{j=1}^{n_i} \left[ y_j - f(x_j,\theta))^T \; C_i^{-1} (y_j - f(x_j,\theta)) \right] + \log (\det Ci) \qquad \text{(eq. 8)}$$

Where $C_i$ corresponds to the individual covariance matrix:

$$C_i = G_i \Omega G_i^T + H_i \sum H_i^T \quad \text{(eq. 8a)}$$

And, $G_i$ is the matrix of partial derivatives of $f_{ij}(\theta,X_{ij})$ corresponding to $\eta$ and H, is the matrix of partial derivatives of $f_{ij}(\theta,X_{ij})$ corresponding to $\varepsilon$ and T is the transpose of the respective matrix.

The objective of the maximum likelihood equation is to make estimates of the fixed parameters ($\theta$) and random parameters ($\Omega$ and $\sum$) of the studied population in such a fashion that the likelihood or probability (L) of observing the observed concentration or effect (Y) is maximized given ( | ) the time ($t_{ij}$), the dose and the mixed effects in question.

## 2.2.2.1 Structural model

Structural models typically describe the input (administration route and absorption or formation of a metabolite) to the physiologic system, the number of compartments in the system (distribution processes) and elimination (metabolism and excretion) from the system (figure 7). In addition, they describe the rate at which molecules of the medicine transfer into the body, between the compartments and out of the body.

PK models make use of the concept of compartments to describe the physiologic system that acts upon the drug after its administration. These compartments are often groups of tissues that are perfused at a different rate, with a central highly perfused compartment that quickly reaches equilibrium and one or more other compartments that are less highly perfused and that reach equilibrium more slowly. A compartment is a region of the body in which the molecules of the medication are well mixed and kinetically homogenous; therefore, they can be represented by a single representative concentration at any time point (147). Differential

equations are used to describe the transfer rates or constants ($k_{xx}$) during absorption, transfer amongst compartments and elimination from the body (figure 7).

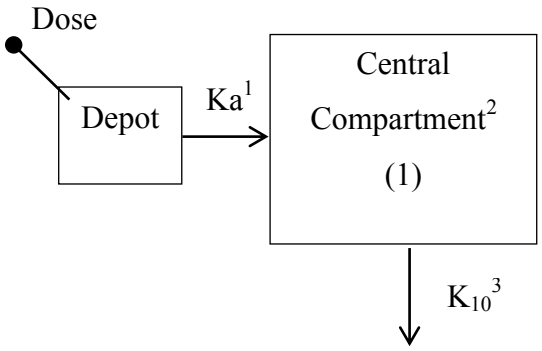


Figure 9. Simple graphic representation of a one compartment structural model for a medicine with oral administration

[1]$k_a$ –dose administration and absorption, bioavailability

[2]Compartment 1 – highly perfused rapidly equilibrating tissues

[3]$k_{10}$ – elimination

Clues about the number of transfer constants can be obtained by using Cartesian and semilogrithmic plots of the plasma concentrations versus time. In orally administered medicines, there is usually an ascending part of the curve which represents the absorption of the medicine. This may be followed immediately by descent of the curve which represents elimination of the medicine. If the descent is monoexponential, meaning the rate of elimination is constant, this usually represents a one compartment model. If the descending curve is bi-exponential it may represent a second compartment that needs to be taken into account in the structural model, this will be reflected in the curve by an inflection. The inflection is often easiest to see in a semilogarithmic plot of the data. An interesting case is that of flip flop kinetics where the constant of absorption ($k_a$) is slower than the constant of elimination ($k_{el}$); this occurs with some extended release medications where there is a plateau in the curve. This represents a period where because of controlled release of the dose from the tablet, the absorption and elimination rates are the same, creating a plateau in the curve. A

good estimation of the elimination rate thus requires to sample sufficiently long to have the absorption phase completed. The elimination rate should be the same as that obtained after I.V. administration.

Structural models are described mathematically by differential equations (eq. 9) and an integral equation (eq. 10).

$$\frac{dC(t)}{dt} = Ka \cdot F \cdot Dose - K_{10} \cdot V_1 \cdot C(t) \qquad \text{(eq. 9)}$$

$$C(t) = \frac{F \cdot Dose \cdot ka}{V1(ka-k_{10})} \cdot (\exp(-k_{10} \cdot t) - \exp(-ka \cdot t) \qquad \text{(eq. 10)}$$

Where C is plasma concentration, t is time, $k_a$ is the constant of absorption, $k_{10}$ is a rate constant for elimination from the central compartment, $V_1$ is the volume of distribution for the central compartment and F is the bioavailability of the dose after oral administration.

The PK model seeks to identify the mean estimate for several important parameters:

- **Clearance (CL)** is defined as the volume of plasma that is completely cleared of a medication per unit of time. It relates to the rate of elimination to the concentration of a medicine in the body.
- **Volume of distribution (V)** is a virtual representation that relates the amount of a medication in the body to the concentration of that medication in the blood. It explains how much of the administered dose is in the circulation versus how much is in other tissues.

$K_{el}$ ($k_{10}$) is the first order rate constant describing drug elimination from the body and is usually derived from CL and V in popPK. This is an overall elimination rate constant describing removal of the drug by all elimination processes including excretion and metabolism.

Bioavailability (F) is another important concept represented in the model, particularly with extravascular administration. It is defined as the fraction of unchanged medicine that reaches the systemic circulation following administration by any route (149). The extent of systemic availability is determined by the extent drug is absorbed from the site of administration and equally by the quantity of medication that avoids intestinal or hepatic first pass elimination. Medications administered intravenously are considered 100% bioavailable while orally administered medicines may be less primarily due to either incomplete absorption or first pass elimination. In the absence of intravenous data in patients in a study, model parameters which are dependent on F for orally administered medications will be termed apparent parameters and denoted as CL/F, V/F, etc.

#### 2.2.2.2 Metabolite kinetics

Information about the PK/PD of metabolites is important, especially in cases, such as tramadol, where metabolites are active. After administration of a parent medicine, formation and disposition of metabolites is governed by metabolite kinetics in the systemic circulation.

Figure 8 is a simplified representation of the metabolism of a medicine. In fact, a parent drug may be metabolised to several metabolites, there may be several sites of metabolism and several routes of elimination, amongst them fecal, respiratory and renal. Additionally, some of the parent drug may remain unmetabolised and be excreted unchanged.

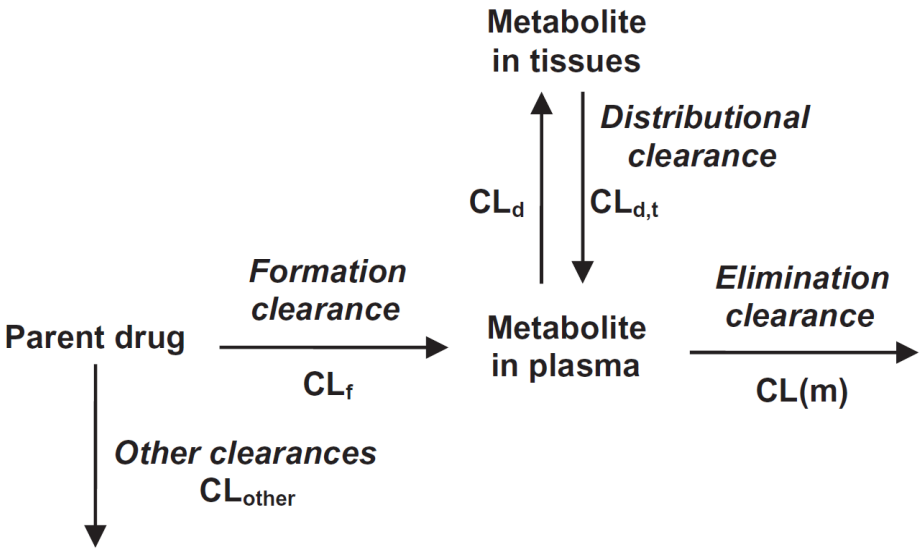


Figure 10. Simple model of metabolite kinetics

$CL_{other}$ – clearance of the parent drug by means other than metabolism, $CL_f$ – clearance of the parent drug that forms the metabolite or formation clearance of the metabolite (may occur in the liver or at other sites of metabolism), $CL_d$ – CL of the metabolite by distribution to the tissues, $CL_{d,t}$ – clearance of the metabolite from tissues to the plasma, $CL_{(m)}$ – total body clearance of the metabolite can be by renal excretion or other methods of excretion such as bile.

Yang Z. In Vivo Metabolite Kinetics. Pharmaceutical Sciences Encyclopedia: John Wiley & Sons, Inc.; 2010 (150) with permission of Wiley.

The following relationship can be used to describe the fraction of the parent drug metabolised

$$\frac{AUC_m}{AUC} = f_m \cdot \frac{CL}{CL_m} \qquad \text{(eq. 11)}$$

Where AUC is the area under the plasma concentration time curve for the parent drug; $AUC_m$ is the AUC for the metabolite; CL is the total body clearance (sum of all means of clearance) and $CL_m$ is clearance to metabolite (150).

For I.V. administration, the amount of unchanged parent drug in urine (eq.12) and amount of metabolite in urine (eq. 13) can be calculated from plasma data:

$$U^{\infty} = \frac{k_e \cdot dose}{k_{el}} \qquad \text{(eq. 12)}$$

$$M_u^{\infty} = \frac{k_m \cdot dose}{k_{el}} \qquad \text{(eq. 13)}$$

Where U is drug in urine; $M_u$ is metabolite in urine; $k_e$ is excretion rate constant; $k_m$ is the metabolism rate constant

## 2.2.2.3 PK/PD modelling approaches

Concentration-effect relationships are central to establishing effective and safe dosing recommendations. The premise that underlies the determination of concentration-effect relationships is that a drug (D) binds to a specific receptor (R) in the body forming a drug-receptor (DR) complex (151). The altered receptor initiates an immediate, delayed or indirect response in the body that is the observable effect of the medicine. The medicine–receptor binding is typically reversible with the net concentration of the medicine at the receptor creating a dynamic balance between binding ($k_{on}$) and dissociation ($k_{off}$) of the medicine. The ratio of these rates, $k_d$, is a constant. Increasing drug concentration, where [ ] represents concentration, reduces free receptors and therefore increases the concentration of DR complexes.

$$\frac{k_{off}}{k_{on}} = k_d = \frac{[D][R]}{[DR]} \qquad \text{(eq. 4)}$$

If [D] is very high, the receptors will be saturated and no additional drug effect will occur. Thus all medications have a maximal effect if the dose is high enough.

Pharmacometricians represent this relationship using a general equation, as follows:

$$E = \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n} \quad \text{(eq.15)}$$

Where E represents effect, C the concentration of the drug, EC50 the concentration at which the effect is 50% of $E_{max}$ and n is a sigmoidicity factor (Hill factor) which describes the steepness of the concentration-effect relationship.

This fundamental concentration-effect relationship assumes that when concentration is zero drug effect is zero. More commonly, there is a baseline pre-drug effect and the relationship between the baseline effect and drug effect needs to be taken into consideration. Common relationships are additive (eq. 16) and proportional (eq.17) (147):

$$E = E_0 + E_{drug} \quad \text{(eq. 16)}$$

$$E = E_0 \cdot (1 + E_{drug}) \quad \text{(eq. 17)}$$

An additive relationship means that the baseline and drug effect have the same slope regardless of baseline throughout the time period evaluated while a proportional relationship means that the slope is dependent on the baseline value throughout the time period evaluated.

In analgesic PK/PD models, placebo effect is an important consideration. The relationship between baseline (see section 2.2.1.2 ***Pharmacodynamics*** for a discussion of baseline), placebo effect and drug effect must be carefully considered. It is commonly assumed that the changes that occur under placebo administration also occur under treatment

administration. Thus efficacy of a drug is the sum of both the true drug effect and the placebo effect. Several options exist to model placebo effect (142), including:

- a linear change in placebo effect over time while the drug effect reduces:

$$E\ placebo = Slope_{pbo} \cdot time$$

$$E_{drug} = \frac{C_p}{EC_{50} + C_p} \qquad \text{(eq. 18)}$$

- an empirical curvilinear relationship:

$$E = Baseline \cdot (1 - E_{drug})\ (1 + E_{pbo})$$

$$E_{placebo} = slope_{pbo1} \cdot time + slope_{pbo2} \cdot time^2 \qquad \text{(eq. 19)}$$

PK/PD data can be fit simultaneously or sequentially. In simultaneous fitting, all data is input to the model directly. In sequential modelling, typically the data for the PK model is fitted and then the PK parameters are fixed. Then the PD data is fitted to the model using the fixed PK parameters. Zhang et alia (152) compared simultaneous and sequential PD analysis and cite simultaneous methods as the gold standard. However, they found that using the First Order Conditional Estimation (FOCE) method, a sequential approach that conditions on both popPK parameter estimates and PK data, estimates PD parameters and their standard errors about as well as the simultaneous method does but saves about 40% computation time.

**Table 4. Population model parameter terms and abbreviations**

| Parameter | | Characteristics | Estimated from the data? * | Description |
|---|---|---|---|---|
| THETA or θ<br>or typical value | | Same for every subject | Y | Fixed effects: Typical value for the parameter |
| ETA or η | | $\eta_i \sim N(0, \omega^2)$ across the population studied | N | Random effects: present for each individual; difference between the population typical value and the individual's parameter value |
| OMEGA or ω | $\sigma^2$ (variance)<br>SD | | Y | $\sigma^2$ or SD of BSV for the parameter across the population being studied |
| EPSILON, ∑ or ε | EPS | $\varepsilon_{ij} \sim N(0, \sigma^2)$ across the population studied | N | Individual predicted residual variability; difference between the individual's observed value and the model prediction |
| SIGMA or σ | $\sigma^2$ (variance)<br>SD | | Y | $\sigma^2$ or SD of EPS difference between observed vs individual prediction |

*Y – yes N- no

## 2.2.2.4 Statistical model

Classical linear regression identifies only 1 level of unexplained variability (i.e. the difference between the model predicted typical value and the particular observation of focus (147). Population models can have two kinds of fixed parameters (see also 2.2.2 Population models and equations 8 and 8a): those that are identified directly from the data rather than being estimated and estimated parameters that incorporate no between subject variability (BSV, also called inter-individual variability (IIV)) (147). Models that include fixed effects and random effects are called mixed effect models and a variety of terms are used to refer to the fixed effect and random effects and the variability associated with them (Table 4.) Population models also include statistical models that are developed to describe the variability around the structural model and fall into the 2 main categories of BSV (variance of a parameter across individuals) and RUV (residual unexplained variability, i.e. variability that remains after all other sources have been controlled for) (153).

## 2.2.2.5 Modeling population variability or ETA

Variability associated with the individual is obtained by adding the typical value for the fixed effect ($\theta$) by the deviation from the population for each subject ($i$) at each time point ($j$).

$$\text{Parameter} = \theta + \eta_{ij} \qquad \text{(eq. 20)}$$

This makes the assumption that the data for the individuals are normally distributed across the population with a mean of 0 and variance of $\omega^2$, expressed as $N(0, \omega^2)$. Often with physiologic data, this is not the case and we may, as with pharmacokinetic data, wish to constrain the values to be positive and right skewed. In this case the data can be log transformed:

$$\text{Parameter} = \theta + \exp(\eta_{ij}) \qquad \text{(eq. 21)}$$

It is important to note that this transformation being executed, the variance estimate ($\omega^2$) is in the log domain and must be converted, as follows, when computing the coefficient of variation:

$$CV(\%) = \sqrt{\exp(\omega^2) - 1} \cdot 100\% \qquad \text{(eq.22)}$$

The results of this operation give an easily understandable reflection of the variability in the population.

## 2.2.2.6 Addition of covariates

Once the structural model is established the data are examined to establish relationships between parameters or individual patient characteristics such as age, sex, glomerular filtration rate (GFR), etc. Visual inspection of the data is an important first step and several plots should be examined to determine whether there is any relationship between a covariable and certain parameters. Relationships revealed in the data plots and likely covariates based on the modeller's knowledge of the pharmacology of the medication and characteristics of the population should be considered for inclusion in the model (147).

One commonly used approach is stepwise covariate screening. In this approach, all covariates to be considered are tested separately and all covariates that meet statistical criteria for inclusion are included in a full model (usually $p = 0.01$). Once the full model is established covariates are then dropped through backward deletion with a stricter criteria. To remain in the model the significance of the covariate must be $p = 0.001$, continuing until all covariates have been re-tested and the final model cannot be simplified further. There is some contention that models using stepwise approaches can be subject to selection bias, overestimating the importance of covariates retained on a statistical basis, although Wahlby et al. (154) reported that the selection bias was small relative to the overall variability in the estimates.

Another approach, advocated recently, is to utilize parametric bootstrap with at least 1000 random samples to determine the bootstrap median and 95% CI for each of the parameter point estimates. Likely covariates such as age, age group and period can then be added to the full model and tested. The influence of the covariate is considered significant if the difference between the means of parameter with and without the covariate fell within the 2.5 to 97.5 percentile bootstrap confidence interval and did not include zero (142, 155, 156).

***Models for residual variability***

Residual variability corresponds to variability that remains after all other sources have been accounted for. There are several accepted including simple additive (eq. 23), proportional (eq. 24) and mixed additive and proportional models (eq. 25) (157).

$$Y = \theta + \varepsilon_1 \quad \text{(eq. 23)}$$

$$Y = \theta \cdot (1 + \varepsilon_2) \quad \text{(eq. 24)}$$

$$Y = = \theta \cdot (1 + \varepsilon_2) + \varepsilon_1 \quad \text{(eq. 25)}$$

Where Y is the concentration or effect, $\theta$ is the typical value for the population for a parameter and $\varepsilon$, the random effects associated with the parameter, which are normally distributed, centred around zero and have a variance of $\sigma^2$. Additive error models are used when the variance is expected to be consistent with time. Proportional error models are used when it is expected that the variance will change and may in fact be several log different (this is particularly relevant for PK). A mixed error model can be used when it is expected that the effect or concentration will in some cases be very small (additive model dominates) and others very large (proportional model dominates).

### 2.2.2.6 Selection and validation of the model

Model evaluation is an important step in pharmacometrics. Different tools are used at different points in the modelling exercise to guide the selection of the most appropriate model.

***Early model development:***

Early in model development the maximum likelihood is an often used tool. With linear regression, the slope and intercept of a line are estimated from the data and then the difference between each value observed for the individual and that predicted ($C_{obs} - C_{pred}$) for the dependent variable is calculated, giving a residual value (147). The best estimation for the parameter is that which gives the lowest value for the sum of squares of the residuals. Similarly, the objective function makes use of the $C_{obs}$ and $C_{pred}$ but, for each pair, $C_{pred}$ has a range of potential values that are normally distributed with a mean of $C_{pred}$ and SD given by $\sigma^2$. The likelihood of the observed data summarises the deviation of the observed data from the centre of the predicted distribution. The Objective Function Value (OFV) is a statistical criteria applied to nonlinear regression models; it measures the difference between the observed and predicted values of parameters and the dependent variable (158). The minimum OFV for a set of parameters (model) and data set is considered to represent the set of parameters that give the best fit for the data. The actual value of the OFV is not important, the OFV is used with models of the same dataset to make comparisons of parameter values and between models for determining goodness of fit (147). The OFV is frequently calculated using first order conditional estimation (FOCE) without or with interaction (FOCE-I) and is a mathematical linear approximation developed by Sheiner and Beal (143).

There are several criterion tools for making these comparisons between models, each of which has its strengths and weaknesses. These include the Aikaike information criteria (AIC) (eq. 26), Bayesian information criteria (BIC) (eq. 27) (142). BIC penalises models with greater complexity (i.e. numbers of parameters) and may be preferable when the data is sparse.

$$AIC = OFV + 2 \cdot n_p \quad \text{(eq. 26)}$$

$$BIC = OFV + n_p \cdot Ln(N) \quad \text{(eq. 27)}$$

Where $n_p$ is the number of parameters in the model and N is the number of data observations.

Keeping in mind that parsimony is a key criteria in selecting a model, it is necessary to consider whether adding complexity by increasing the number of parameters truly adds value in describing the data and system. A model with a lower OFV and more parameters can be a near perfect description of the data but it may also be overfitted and describe noise rather than the underlying relationship (142). When this happens, the model cannot be reliably used for prediction between data points or for extrapolation outside the range of the data. Although parsimony is a key consideration in model selection, mechanistic plausibility and utility are more important than a small OFV(142).

***Validation of the final model***

The likelihood ratio test (LRT) can be used to compare the OFV of two models and assigns a probability that they provide the same description of the data; one model must be a subset of the other and have a different number of parameters (i.e. they must be nested). Typically, when comparing a base model and others, the model which gives the lowest value of likelihood function is considered the optimal model (147). Thus LRT, the difference in the log-likelihoods (or the log of the ratio of both likelihood) is useful in comparing covariate models and base models (eq. 28) (159).

$$LRT = -2 \cdot \ln \left( \frac{\text{Likelihood for the base model}}{\text{Likelihood for the alternative model}} \right) \quad \text{(eq. 28)}$$

LRT follows a $\chi^2$ distribution with the number of degrees of freedom equal to the number of additional parameters. The significance of the difference between the models can be tested using the difference, significance level desired and the difference in the number of parameters between the two models. So, if we set our significance at $\alpha = 0.05$, in where the alternative model has one additional parameter (thus one degree of freedom), the LRT will need to be 3.84 to make the determination that the increase of 1 parameter adds statistically significant value to the model.

Graphical evaluations are another way of examining model fitness. Classically, the individual observed, population predicted and individual predicted against time are plotted. The population prediction should reflect the typical patient and so be centred in the pooled observed data (Figure 9a) while the individual predictions should closely follow the observed data (Figure 9b). Plotting of the weighted residuals (WRES) is also important. Residuals should be weighted so that the SD is 1. Weighted residuals give us information about the deviation between the model predictions and the observed data. WRES plotted against time give us information about the adequacy of the structural model and should be evenly centred around zero with no systematic bias and most values between ± 2 SD (Figure 7c). Plots of the WRES against population predicted values should be evenly centred around zero, without systematic bias and with most values ± 2 SD; systematic deviations could reflect deficiencies in the statistical models for RUV (Figure 7d) (142).

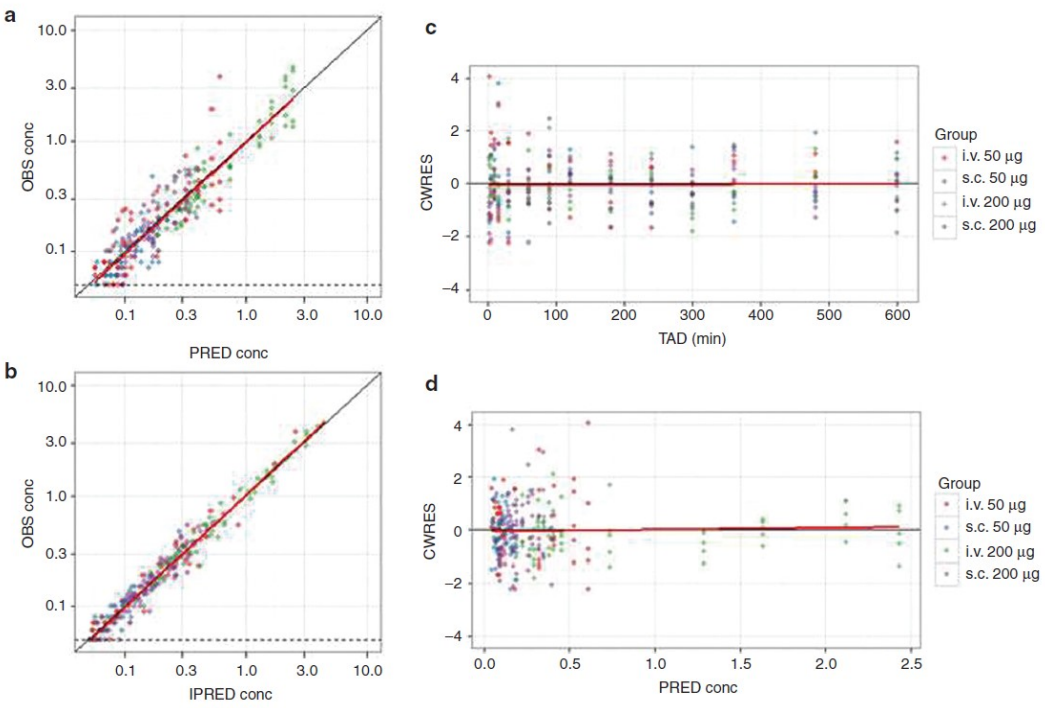


Figure 11. Sample diagnostic plots for graphical evaluation of an imaginary PK model a) observed concentration ($OBS_{conc}$) versus population predicted concentration (PRED), b) $OBS_{conc}$ versus individual predicted concentrations (IPRED), c) conditional weighted residuals (CWRES) versus time after dose (TAD), and d) CWRES versus population predicted (PRED) concentrations (142) with permission of Creative Commons.

Another important aspect of validating the final model is to test the stability of the model. This is usually done by means of a bootstrap technique (160). Furthermore, bootstrapping permits us to determine the precision and standard error of the parameter estimates (161). Bootstrapping is a resampling technique that involves generating at least 1000 replicate datasets where individuals are randomly drawn from the original datasets and can be redrawn multiple times or not at all for each replicate (142). Many replicates are generated and evaluated using the final model to ensure that the parameter distributions reflect the parameters of the original dataset. Bootstrap percentiles can then be constructed by taking the lower 2.5% and upper 97.5% parameter estimate. If the parameter estimate fits within the 2.5-97.5 percentile bootstrap CI, then the estimate is considered adequately precise.

Finally, visual predictive check (VPC) plots are used to demonstrate model performance. These plots are constructed by simulating data from the original or a new database and using the final model to simulate concentration time profiles and prediction intervals (usually with the 95% CI) and compare them with the observed data. The plot should show the majority of the predictions and observed data being within the 95% CI over the concentration-time profile.

# Chapter 3: Objectives and research hypothesis

The complexity of using analgesics in elderly persons cannot be underestimated, key considerations that affect PK and PD include age-associated changes in body composition and function. PK and PD data on analgesics in elderly patients, especially those aged >75 years, are sparse (1-3, 10), even in analgesics commonly used to treat painful conditions which occur frequently in the elderly (15). The overall objective of this research program is to provide information on the PK and PD of both tramadol, a weak opioid analgesic widely used in the elderly, and its active metabolite ODM in subjects 75 years and older in order to determine whether there are age related differences.

The analyses presented in this thesis are intended to meet this objective. They are conducted on the results of a single randomised, double-blind, placebo-controlled, crossover study in healthy 20 young (aged 18-40 years) and 15 elderly subjects (aged 75 years and older). Subjects in the study were randomly assigned to a crossover treatment sequence where they received a single 200 mg dose of tramadol administered as a once-daily extended release tablet on one occasion and a placebo tablet that was identical in appearance on the other occasion. There was a 7-day washout between treatment occasions. Two-cohorts were enrolled one January 2007 and one in February 2007.

The dosing and evaluation phases of the study were identical for the two administration periods and for both cohorts. Two kinds of PD evaluations were conducted (i.e. Current Perception Threshold, Pain Perception Threshold (PPT) and Pain Tolerance Threshold (PTT)) using electrical stimuli at 250 Hz and 5 Hz. On the evening prior to the first dose, subjects received training during which they had the electrical stimulus procedure explained to them. They also had at least 2 practice procedures for each of the CPT and PTT procedures to minimize bias in the CPT and PTT levels at the early time-points due to a learning effect. PD evaluations were performed at immediately prior to dosing and at 15 time-points post dose (0.33, 0.75, 1.25, 1.75, 2.5, 3.5, 4.5, 5.5, 7, 9, 11, 14, 20, 24 and 30 h post-dose).

During the conduct of the study, blood samples were collected immediately prior to dosing and 16 post dose time points (0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 20, 24, 30, 36, 42 and 48 h). Complete urine output was collected from 4 h prior to dosing and for 48 h post dose. All samples were analysed for (+)- and (-)-tramadol and (+)- and (-)- O-desmethyltramadol (ODM).

The manuscripts presented in this thesis accomplished the overall objective as follows:

- The first manuscript characterises the PK of both enantiomers of tramadol and O-desmethyl tramadol in healthy young (18-40) and elderly subjects (75 years and older) using two analyses:
    - A non-compartmental analysis that examines the stereoselective PK of (+)- and (-)-tramadol and (+)- and (-)-ODM in plasma and urine;
    - A popPK analysis that aims at identifying covariates such as age, sex, glomerular filtration rate (GFR), and food-effect (FE) that might result in differences in disposition, metabolism and elimination in elderly subjects as compared to young subjects.

- The second manuscript presents exploratory analyses of data from a study utilising an ESPM. The objective of this work was to assess differences between young and elderly subjects with regard to pain tolerance of transcutaneous electrical stimuli at 250 Hz and 5 Hz in order to:
    - determine whether the ESPM utilised in the study is able to detect a difference in elderly and young subjects at 5 Hz and 250 Hz after a single dose of placebo and tramadol.
    - select the most reliable frequency for the (+)-ODM PK PD analysis presented in the third paper.

- The final manuscript presents a pop PK/PD analysis which had as its objective the description of any age related differences in the PK/PD of (+)-ODM, the active metabolite of tramadol, using the PTT as a biomarker for analgesic effect.

# Chapter 4: Pharmacokinetics of Tramadol and O-Desmethyltramadol Enantiomers Following Administration of Extended-Release Tablets to Elderly and Young Subjects (Manuscript 1 – Published in Drugs and Aging )

## 4.1 Introduction

The purpose of this article was to present the data characterisation of the PK of both enantiomers of tramadol and O-desmethyl tramadol in healthy young (18-40) and elderly subjects (75 years and older). This work represents the most extensive characterisation of these PKs in elderly subjects, 75 years and older. We presented the data using two analyses a non-compartmental analysis that examines the stereoselective PK of (+)- and (-)-tramadol and (+)- and (-)-ODM in plasma and urine and a popPK analysis of racemic Tramadol that aims at identifying covariates such as age, sex, glomerular filtration rate (GFR), and food-effect (FE) that might result in differences in disposition, metabolism and elimination of tramadol in elderly subjects as compared to young subjects. This article laid the ground work for the subsequent population PK/PD analysis of (+)-ODM.

Sybil Skinner-Robertson made substantial contribution to study conception and design, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content; and final approval of the version to be published. Her contribution to the writing of the manuscript is estimated at 75%, Dr. Varin and Dr. Mouksassi having made an estimated contribution of 20% and Drs Bouchard and Fradette having contributed 5%.

*The final publication is available at Springer via http://dx.doi.org/ 10.1007/s40266-015-0315-4"*

## 4.2 Manuscript

Pharmacokinetics of Tramadol and O-Desmethyltramadol enantiomers following Administration of Extended-Release Tablets to Elderly and Young Subjects.

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Key Points:

Differences in tramadol pharmacokinetics between relatively healthy elderly volunteers and young healthy volunteers are not remarkable after a single dose.

No differences in PK parameters related to the absorption process are observed.

Elderly subjects have a slower elimination rate constant and age-dependent increase in V/F

Food-effect associated with higher peak plasma concentrations of tramadol is more frequent in young subjects,

Exposure to ODM is higher in relatively healthy elderly subjects versus young healthy subjects.

Elderly subjects have a slower elimination rate constant that is mostly explained by a reduction in renal clearance

This is important since ODM+ is postulated to be primarily responsible for the opioid analgesic effect and opioid side effects associated with tramadol administration.

Use of tramadol in elderly populations and particularly in the more frail elderly should carefully consider the patient's renal and hepatic function and the increased potential for opioid related side effects.

**ABSTRACT**

**Background:** Tramadol (T) is frequently used in geriatric patients; pharmacokinetic (PK) publications on T and O-desmethyltramadol (ODM) in elderly are rare.

**Objective:** Characterization of T and ODM PK, including absorption processes and covariates for tramadol in elderly and young subjects after single dose administration of 200 mg extended-release tablets

**Methods:** A PK study of 14 elderly (≥75 years) subjects with mild renal insufficiency and 34 young (18-40 years) subjects was conducted with blood and urine samples collected for 48 hours post-dose. Noncompartmental Analysis (NCA) of each T and ODM enantiomer included: area-under-the-concentration-time curve (AUC), terminal elimination rate ($k_{el}$), total body clearance, volume of distribution ($V_{area}/F$) and renal clearance ($Clr_{0-48}$). A one compartment population model of total tramadol concentration was parameterized with clearance (CL/F), volume of distribution (V/F) and mixed order absorption (first-order and zero-order absorption rate constants with lag times).

**Results:** NCA demonstrated comparable $C_{max}$ and AUC between age-groups for T enantiomers, but significant differences in $V_{area}/F$ (mean 34% higher) and $k_{el}$ (mean 28% lower) in the elderly. ODM PK were significantly different in the elderly for $AUC_{0-inf}$ (mean 35% higher), $Clr_{0-48}$ (mean 29% lower) and $k_{el}$ (mean 33% lower). The population analysis, identified age as a covariate of V/F (Young: 305 L; Elderly: 426 L) with a 50% longer mean elimination half-life in the elderly. No differences in absorption processes were observed.

**Conclusions:** Tramadol exposure was similar between the age-groups; exposure to ODM was higher in elderly subjects.

# 1 INTRODUCTION

Research on medicinal treatments used in elderly patients is lacking, despite aging of the population and the increase in chronic medical conditions, globally. A search of the Clinicaltrials.gov data base revealed that in 2010, of the 1545 clinical trials conducted in central nervous system (CNS) indications, only 1.5% included patients older than 75 years. Furthermore, less than 10% of drug delivery technology trials conducted included Pharmacokinetic (PK) assessments in the elderly. Although pain is prevalent among the elderly, PK and Pharmacodynamics (PD) data on analgesics in elderly patients, especially those older than 75 years, is sparse, yet this data is critical to ensure safe use of these medications in this population (1-5).

Tramadol hydrochloride is a widely used centrally acting analgesic that binds to mu-opioid receptors and also inhibits serotonin and norepinephrine reuptake in the descending inhibitory pain pathways(5, 6). Tramadol is indicated in the treatment of moderate to severe pain in a variety of pain conditions and used widely in the elderly population (7-10). Once-daily formulations of tramadol that reduce plasma concentration variability and improve consistency of drug delivery(11) have been developed and are useful in chronic pain conditions that affect the elderly (3, 8, 12). One of these, a tramadol extended-release tablet, was administered in this study.

Tramadol, a racemic 1:1 mixture of (+)-tramadol and (-)-tramadol, is rapidly and extensively biotransformed in the liver via CYP2D6, CYP3A4 and CYP2B6, resulting in the formation of 11 metabolites. The two major ones are O-desmethyltramadol (ODM), the primary active metabolite (13, 14) and mono-N-desmethyltramadol (5, 6). Phase II reactions, mainly conjugation of 0- and N-desmethylated compounds result in the formation of an additional 12 metabolites. Genetic polymorphisms in CYP2D6 contribute to the variability seen in the PK and PD of tramadol (15-17). In young subjects, after hepatic biotransformation, tramadol and its metabolites are largely eliminated by the kidneys (~90%) with the remainder eliminated in feces. Approximately 12-25% of an oral dose of tramadol is excreted unchanged in urine, while ODM (15%) and its conjugates, M2 and M5, are the main metabolites (6, 18).

Age-related changes in hepatic and renal function have the potential to affect the PK of tramadol resulting in altered efficacy and safety in elderly patients that may necessitate dose adjustment

(1, 3, 19, 20). Brouquet et al. (21) found that tramadol administration was a risk factor for post-operative delirium in patients over 75. Concerns continue to be expressed about the use of tramadol in elderly patients, particularly those over 75 years.

The present study was conducted in healthy elderly (75 years and older) and young volunteers (18-40 years) to determine whether differences exist between these age-groups with regard to single-dose PK of 200 mg tramadol extended release (ER) tablets intended for once daily administration. The tablets, composed of immediate-release (IR) and controlled-release (CR) matrices, were designed to attain therapeutic tramadol plasma concentrations within 2 hours of administration and provide continuous drug delivery over 24 hours following a single dose. Relative bioavailability studies of this formulation compared to immediate-release formulations in healthy young volunteers demonstrated 95% relative bioavailability , reduced peak plasma concentrations and a terminal elimination half-life of 6.5 (ER) versus 6 (IR) hours (22-26).

Two analyses of the PK data are presented in this paper: a non-compartmental analysis which examines the stereoselective PK of (+)- and (-)-tramadol and (+)- and (-)-ODM in plasma and urine; and a population PK analysis which aims at identifying covariates such as age, gender, glomerular filtration rate (GFR) and food-effect (FE) that might result in differences in disposition, metabolism and elimination in elderly subjects as compared to young subjects

## 2 MATERIALS AND METHODS

### 2.1 Experimental design

A two-cohort, PK study was conducted at a phase 1 facility where subjects were confined for 12 hours prior to dosing and for 48 hours afterwards. This study, conducted between January and February 2007, was intended to evaluate the PKs of Tramadol Contramid® ER tablets (T) in elderly (≥ 75 years) and healthy young (18-40 years) volunteers .

### 2.2 Subjects

At screening, subjects were determined to be healthy based on medical history, physical examination, and evaluation of vital signs, electrocardiogram (ECG), and clinical laboratory data. Subjects were genotyped and those with the poor metaboliser variant of the CYP2D6 gene were excluded to minimize intra-group variability and inter-group differences not related to age. Potential subjects with a body mass index (BMI) > 35 $kg/m^2$ or those who had donated blood

frequently in the previous year were excluded: as were subjects with an increased risk of seizures, with bowel disease affecting absorption or previous failure of treatment with tramadol or discontinuation of treatment due to adverse events. Female subjects of childbearing potential had to have negative pregnancy test results at screening and clinic check-in for each study period. Subjects abstained from taking substances known to be strong inhibitors of CYP isoenzymes within 10 days, or inducers of CYP isoenzymes within 28 days, prior to dosing. Use of all medication (including over-the-counter products) was prohibited for 7 days prior to dosing and during the time of sample collection with two exceptions: elderly subjects were permitted to continue taking stable doses of chronic medications, other than strong CYP inhibitors/inducers, and female subjects were permitted to continue taking hormonal contraception or replacement therapy. Use of concomitant medications was recorded.

### 2.3 Evaluations and pharmacokinetic sampling

#### *2.3.1 Sample collection*

Blood samples were collected prior to the time of dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 20, 24, 30, 36, 42 and 48 hours post-dose. Blood samples were collected by direct venipuncture or butterfly catheter into lithium heparinated collection tubes. Samples were immediately cooled in an ice-bath and centrifuged under refrigeration. Plasma samples were then divided into two aliquots and stored at -20±10°C, pending assay.

Urine samples were collected prior to dosing (-4 to 0 hours) and at the following intervals after dosing: 0-8, 8-12, 12-24 and 24-48 hours. During the collection period, urine samples were pooled and refrigerated. Total volume collected for each interval was recorded and two 5 mL aliquots stored at below –20°C until analysis.

#### *2.3.2 Bioanalytical method*

Plasma and urine concentrations of tramadol and ODM-metabolite enantiomers were measured by high performance liquid chromatography (Agilent 1100) using a Chiralpak® IA (250x4.6 mm, 5µm) analytical column maintained at 5°C. The mobile phase consisted of acetonitrile:water:diethylamine (950:50:0.1; v/v/v) delivered at a rate of 0.6 ml/min. Elution times were within 9 min for all analytes. A triple quadrupole mass spectrometer (Applied Biosystems, API 4000) at unit resolution in the multiple-reaction-monitoring mode was used to

monitor the transition of the protonated precursor ions to the product ions by the Turbo V electrospray interface (ESI). Transitions were *m/z* 264 to *m/z* 58 for tramadol enantiomers, and *m/z* 250 to *m/z* 58 for ODM enantiomers. Main parameters were the following: source temperature 650°C, ion spray voltage 5.25 kV, declustering potential 46 V. MS/MS parameters were: collision energy 51 eV, collision gas pressure (N2) 6 mPa.

Plasma (0.3ml) and urine (0.2ml; previously deconjugated by 1h-incubation at 37°C with 537 B-glucuronidase units) samples were vortexed after successive addition of internal standard (ketamine) and 1M sodium carbonate buffer (0.1ml, pH9); then extracted with 3 ml of hexane:chloroform (3:2) by vortexing for 5 minutes before centrifugation. The aqueous phase was flash-frozen in an alcohol bath (-18°C) and the organic phase decanted, evaporated to dryness under nitrogen at 40°C and reconstituted with 5 ml of acetonitrile:water:diethylamine (50:50:0.1;v/v/v) by vortexing for 30 seconds. Injection volume was 5 ul.

The method was validated by FARMOVS-PAREXEL Clinical Research Organisation, (Bloemfontein, South Africa) according to procedures and acceptance criteria recommended for bioanalytical method validation for pharmacokinetic studies (27). Matrix effects were lower than the linear range. For plasma, calibration curves fitted a Wagner regression over the ranges of 3.126-400.1 ng/ml for (+)- and (-)-T and 1.563-200.0 ng/ml for (+)- and (-)-ODM. For urine, calibration curves fitted a Wagner regression over the ranges of 66.71-8512 ng/ml for (+)- and (-)-T and 95.82-12227 ng/ml for (+)- and (-)-ODM. For both plasma and urine, mean efficiencies of extraction were 81% (CV 3.1%) and 66% (CV 3.2%) for tramadol and ODM enantiomers, respectively. For plasma, mean inter-day accuracy ranged between 98.2 and 102.0 % with a maximum CV for precision of 6.9% for all analytes. For urine, respective values ranged between 94.8 and 102.9 % with a maximum CV for precision of 12.2 % for all analytes.

### 2.4 Non-compartmental analysis

#### *2.4.1 Calculation of plasma pharmacokinetic parameters*

PK parameters for (+)- and (-)-tramadol and, where appropriate, for (+)- and (-)-ODM, were derived using standard noncompartmental methods with PhAST 2.3-001 (MDS Pharma Services, Montreal, Canada). The apparent terminal elimination rate constant, $k_{el}$, was obtained by log-linear regression of the plasma concentration-time curve (using three or more non-zero data points). The area under the plasma concentration-time curve from time zero to the time

corresponding to the last measurable concentration ($AUC_{0-t}$) or up to infinity ($AUC_{0-inf} = AUC_{0-t} + C_{last}/k_{el}$) were calculated by the linear trapezoidal method. Apparent total body clearances (CL/F) of (+)-tramadol and (-)-tramadol were calculated as $Dose/AUC_{0-inf}$. Apparent volume of distribution was calculated as $V_{area}/F=Dose/(AUC_{0-inf}*k_{el})$. Glomerular filtration rate (GFR) was calculated using serum creatinine according to the Chronic Kidney Disease Epidemiology Collaboration formula: GFR = 141 X $\min(S_{cr}/\kappa,1)\alpha$ X $\max(S_{cr}/\kappa,1)$-1.209 X 0.993 age X 1.018 [if female] X 1.159 [if black]; where $S_{cr}$ : serum creatinine (mg/dL), κ: 0.7 for females and 0.9 for males, α: –0.329 for females and –0.411 for males, min: minimum of Scr/κ or 1, and max: maximum of Scr/κ or 1 (http://www.qxmd.com/calculate-online/nephrology/ckd-epi-egfr) (28, 29).

### *2.4.2 Calculation of 48 h urine collection pharmacokinetic parameters*

The amount of drug excreted for each collection interval ($Ae_{t'-t''}$), was calculated by multiplying the parent drug or metabolite concentration by volume. The total amount excreted in urine over the entire 48h period ($Ae_{0-48}$) was obtained by adding the amount excreted over each interval. For renal clearance (CLr /F), the amount excreted over 48 hours was divided by the AUC calculated over the same period. For some subjects, this required extrapolation of BLQ plasma concentrations which was done using the terminal slope according to the following equation: $(\exp(\ln C_{last})-(t_2-t_1)*k_{el})$. Metabolic clearance was calculated (molar/molar) as $Ae/AUC_{0-48}$*wt. The metabolic ratio (molar/molar) of ODM over the parent compound was calculated by dividing their respective $Ae_{0-48}$.

### *2.4.3 Statistical Analyses*

Data for $C_{max}$, $T_{max}$, $k_{el}$, Cl/F, $V_{area}/F$, $Ae_{0-48}$ and $CLr_{0-48}$ are presented using descriptive statistics (mean, Standard Deviation (SD) and Confidence intervals (CI)) or median when appropriate. Analyses of variance (ANOVA) were performed on the ln-transformed AUC, $C_{max}$ and untransformed $t_{½}$, CL/F, $V_{area}/F$, $Ae_{0-48}$ and CLr. A nonparametric analysis (Wilcoxon rank-sum test) was performed on $T_{max}$. The ANOVA model included age, group, period (group) and the interaction term age*group as fixed effects (subsequently removed for all PK parameters except $CL_{0-48}$ for (+)-tramadol).

Statistical analyses were conducted using paired t-tests to explore differences between enantiomers within the age-groups and two-way ANOVA for age-related differences. The ratio

of the difference between the young and elderly was also calculated. These ratios and the corresponding confidence intervals were expressed as a percentage relative to young and it was to be considered that there was no difference between the age groups if the 90% CI fell within the range of 80-125%. Statistical testing was performed using SigmaPlot version 11.0 (SysStat, San Jose, CA) and descriptive stats were generated using Excel 2010.

**2.5 Population PK Analysis**

Individual plasma concentrations of both (+)- and (-)-Tramadol were added at each time point to obtain total tramadol concentrations. Population PK analysis was conducted using NONMEM® 7.2.0 (ICON, Ellicott City, Maryland). First-order conditional estimation (FOCE) methods with or without interaction were used to test convenient population PK models. S-PLUS 8.0 for Windows (Insightful Inc., Seattle, WA) and Sigma Plot were used for the visual inspection, goodness-of-fit and graphic display of data.

The structural model was parameterized in terms of CL/F, V/F and absorption rate constant (ka), duration of zero-order input ($D_2$) (calculated from the zero-order absorption rate ($ka_0$)), lag time before first-order absorption (LAG1), fraction of the dose absorbed by a first-order process ($f_1$) and lag time before zero-order absorption (LAG2). Combined zero- and first-order absorption inputs were utilized to reflect the immediate and controlled release portions of the tablet, respectively.

Between-subject variability of the pharmacokinetic parameters was characterized using the exponential error model:

$$Pi = TVP * EXP(ETAi)$$

where ETAi is the proportional difference between the hypothetical parameter estimate of the ith subject (*Pi*) and the typical population parameter value (TVP) and assumed to be normally distributed with a mean of 0 and a variance of $\omega i^2$.

Residual error was evaluated using an additive and proportional error model:

$$Y_{obs} = Y_{pred} + Y_{pred} * \varepsilon(1) + \varepsilon(2)$$

Where $Y_{obs}$ is the observed plasma concentration and, $Y_{pred}$ is the model predicted plasma concentration and ε(k) is a normally distributed parameter with a mean of 0 and a variance of $\omega_k^2$.

The first-order conditional estimation method with interaction between variability and residual variability was the primary method used throughout the model building exercise.

Food-effect was explored since five potentially influential individuals were identified during visual inspection. Determination of whether single individuals were influential, was done according to the jackknife procedure (30) which eliminates individual subjects from the model one by one. Parameters estimates and SE% were assessed for a ± 20% difference in the minimum objective function value (OFV).

A routine covariate analysis was conducted on susceptible covariate-parameter pairs using the power model approach. Covariates were added in a stepwise fashion if addition resulted in a decrease in OFV of 3.84 ($\chi^2$, $p< 0.05$; 1 degree of freedom) and if the log-likelihood ratio test met the significance level $\alpha = 0.05$. Stepwise backward elimination was performed using the criteria of 6.63 ($\chi^2$, $p< 0.01$; 1 degree of freedom). Visual inspection of diagnostic plots was performed at each step on the estimated variability of parameters and available individual subject covariates, such as age-group (categorical: elderly=1, young=0), sex (categorical: male=1, female =0), weight, BMI and GFR (28, 29). Diagnostic goodness-of-fit plots were used throughout the model building process to help guide model selection.

Attempts to include the potential food-effect in the model took two different approaches: first, categorically designating a particular subject as having a food-effect (1) or not (0) and second, by designating the particular time points in the subject's concentration-time profile as food-effect (1) or not (0) (30).

To evaluate the model suitability to simulate data, final model parameters were used to simulate the observed dataset 1000 times and observed versus predicted quantiles were computed and compared visually. To assess the model robustness and stability a nonparametric bootstrap analysis was conducted with 1000 replicates where subjects stratified by age-group were resampled with replacement and final model refitted to the resampled data. A 95 % confidence interval was computed using the percentiles method (31, 32). Steady-state predicted AUC, $C_{max}$,

and $t_{1/2}$ were computed using Phoenix® WinNonlin® version 6.4 (Certara L.P. (Pharsight), Princeton, NJ)

**2.6 Safety assessment and analysis**

Seated blood pressure and heart rate were measured before dosing and at approximately 2, 4, 6, 8, 10, 14, 16 and 24 hours following drug administration. Subjects were monitored throughout confinement for adverse reactions to the study formulations and/or procedures. Serum chemistry and hematology, urinalysis, physical exam and 12-lead ECGs were performed at screening and end of study.

Adverse events were coded using MedDRA version 9.1 and summarized by treatment for the number of subjects reporting the adverse event and the number of adverse events reported. A by-subject adverse event data listing including verbatim term, coded term, treatment group, severity, and relationship to treatment was provided.

3 RESULTS

**3.1 Subjects and safety analyses**

Thirty-five (35) subjects were enrolled: 15 subjects aged 75 years or older and 20 subjects between 21-40 years old. One elderly subject was excluded from data analysis because of early discontinuation for personal reasons. Two cohorts of 19 and 15 were enrolled approximately one month apart. Demographic characteristics of study participants are presented in Table 1. The proportion of females enrolled was similar in each age-group. At baseline, elderly and young subjects were significantly different ($p< 0.001$) with regard to GFR and BMI.

There were 118 adverse events reported in 23 of 35 subjects (65.7%) during this study. All adverse events were mild (72.9%) or moderate (27.1%) in severity. Sixteen subjects (7 elderly and 9 young) reported 97 adverse events that were considered possibly or probably related to study medication. Adverse events had resolved by the end of the study, except for one mild episode of pruritus experienced by a subject who was lost to follow-up. No serious adverse events were reported, nor did any subject withdraw because of adverse events. The most commonly (>10 %) reported adverse events are presented by age-group in Table 2. No clear relationship was observed between, $C_{max}$, $T_{max}$ or AUC and occurrence of side effects.

All mean vital sign variables were within normal limits. Mean serum chemistry, hematology and urinalysis variables remained within the reference range at the end of the study.

### 3.2 Noncompartmental Pharmacokinetics

#### *3.2.1 Tramadol enantiomers*

Mean plasma concentration-time profiles of (+)- and (-)-tramadol after single oral administration of tramadol ER 200 mg tablets are shown in Figure 1 (upper panels) and non-compartmental PK parameters presented in Table 3. Maximum plasma concentrations were reached at a median of 6-7 hours post-dose for both young and elderly subjects. Thereafter, plasma concentrations declined more slowly in the elderly than in young subjects.

In each age-group, there was a similar mean extent of systemic exposure, characterized by AUC and $C_{max}$. Accordingly, CL/F was similar between elderly and young subjects for both (+)- and (-)-tramadol. However, $k_{el}$ and Clr were statistically lower and $V_{area}/F$ statistically greater in elderly subjects.

The mean percentage of unchanged (+)-tramadol excreted in urine over 48 hours was similar in both groups: 20% and 18% in elderly and young subjects, respectively. The corresponding values for (-)-tramadol were 17% and 16%, respectively. (Table 3)

A within age-group comparison of enantiomers demonstrated a statistically higher AUC for (+)-tramadol associated with a lower $V_{area}/F$, $k_{el}$ and CL/F when compared with (-)-tramadol ($p< 0.001$ in all cases). The difference for $CLr_{0-48}$ was not statistically significant between the tramadol enantiomers in either age-group but the $Ae_{0-48}$ was significantly higher for (+)- and (-)-T.

#### *3.2.2 O-Desmethyltramadol enantiomers*

Mean plasma concentration-time profiles of (+)- and (-)-ODM are shown in Figure 1 (lower panels) and PK parameter data are shown in Table 4. Maximum plasma concentrations of (+)- and (-)-ODM were reached at a median of 7-11 hours post-dose for both groups. Thereafter, plasma concentrations declined more slowly in the elderly than in young subjects. $AUC_{0-inf}$ was

approximately 1.3-fold higher in elderly subjects for both enantiomers. Mean Clr and $k_{el}$ were statistically lower in the elderly for both enantiomers by approximately 30%. Total amount of (+)- and (-)-ODM excreted in urine over 48 hours was similar in both groups. CLr was statistically different for (+)- and (-)-ODM and $k_{el}$ was different for (+)-ODM in the young age-group but not in the elderly.

Metabolic clearance of (+)-T to (+)-ODM in the young was 0.07 (90% CI: 0.06 - 0.08) and in the elderly 0.05 (90% CI: 0.04 – 0.06). The difference was not significant. Metabolic clearance of (-)-T to (-)-ODM in the young was 0.05 (90% CI: 0.04-0.06) and In the elderly was 0.04 (90% CI: 0.03-0.05). The mean urine metabolic ratio (+)-ODM to (+)-T to was 0.70 (90% CI: 0.60-0.70) in the young and 0.70 (90% CI: 0.53-0.87) in the elderly. For the ratio of (-)-ODM to (-)-T to in the young it was 0.59 (90% CI: 0.52-0.66) and in the elderly it was 0.62 (90% CI: 0.49-0.75).

3.3 Population pharmacokinetic model

***3.3.1 Model Development for tramadol***

Population models reported for tramadol in young healthy volunteers were first identified: a single (33) or a two-compartment (34) model, both of which proposed zero- and first-order inputs with a lag time. As a result, one and two compartment structural models were considered during model development. The former was identified as the most appropriate to fit the data since V/F estimates for a second compartment were too small to be of any physiological relevance. Therefore, in the interests of parsimony, the second compartment was not included in the model. Table 5 presents key steps in the chronology of structural and covariate model development.

Due to the IR and CR design of the formulation (Figure 2), mixed-order absorption (MOA) structural models with corresponding lag times were used to describe the disposition of tramadol after administration of this formulation. Two (2) MOA, one compartmental models were evaluated. In one model, the proportion of first-order and zero-order absorption were estimated while in the other, the fraction of dose absorbed by a first-order process ($f_1$) was fixed to 25% to reflect the formulation design. Sensitivity analyses determined that a one-compartment model with unfixed mixed first- and zero-order absorptions best represents the disposition of tramadol after administration in our population. A full block variance-covariance matrix was used to

describe the covariance between V/F and CL/F. Between-subject-variability was estimated for ka and $f_1$. After routine covariate analysis, only age-group as a covariant on V/F was added.

Comparison of observed versus population predicted and observed versus individual predicted tramadol plasma concentrations (35) were conducted (Figure 3). These tests supported the selection of this model as the most appropriate for the data. Visual examination of the individual PK curves identified that 5 of the 34 subjects exhibited a more rapid absorption profile and significantly higher $C_{max}$ than the 29 other subjects (Figure 4). Demographics revealed that 4 of them were young and 1 subject was elderly. Other characteristics examined included, sex, weight, BMI, GFR, smoking status, adverse events and concomitant medication revealed no consistent similarities between the subjects.

Adding a food-effect as a covariate on each of the parameters resulted in worsening of the OFV, failure of the model to converge, zero gradients or implausible parameters. In no case did excluding an individual from the analysis result in a change in objective function or parameter estimates sufficient to meet standard criteria for considering an individual as influential. However, when all 5 of the individuals (85/513 plasma concentration data points) exhibiting a food-effect were removed simultaneously and the model was run with the remaining 29 subjects (16 young, 13 elderly), the objective function improved dramatically (from 2291.91 to 1836.54) (Table 5). Standard diagnostic plots of the final model with all patients and with food-effect patients removed are presented in Figure 3. Mean estimates for the PK parameters generated by the population model including apparent volume and age as covariates, but excluding the food-effect subpopulation, are presented in Table 6. The model generated a larger apparent volume for elderly subjects (426 L) than for young subjects (305 L).

The steady-state pharmacokinetic parameters based on the simulations supported the single dose findings. $AUC_{0\text{-}tau}$ was similar: 5017 ng*h/mL (95% CI: 4931, 5103) in the young and 5015 ng*h/mL (95%CI: 4929, 5101) in the elderly. However, $C_{max,ss}$ (young: 294 ng/mL (95% CI: 289, 299); elderly: 269 ng/mL (95% CI: 264, 274)) and half-life (young: 8h (95% CI: 7.88 , 8.12 ); elderly: 11h (95% CI: 10.83, 11.17) showed a greater difference which is expected given the age effect on apparent volume seen in single dose PK. Accordingly, accumulation index was higher in the elderly (1.28) versus the young (1.14).

### *3.3.2 Model Evaluation*

The final model obtained with the original dataset was subjected to a bootstrap analysis. Results are detailed in Table 6 which shows that median parameters obtained from the bootstrap were close to the estimates obtained with the original dataset as evidenced by percent difference < 10% for all POP PK parameters. Furthermore, all final model parameters were within the 95% confidence interval for the bootstrap. Visual predictive checks (VPC) demonstrated that the final model simulated values were consistent with the observed data (Figure 5). The observed median (solid red line), and 5th and 95th percentiles (dashed red lines) were captured by the corresponding 95% confidence interval of the simulated prediction intervals (red shading for the median, blue shading for the 5th and 95th percentiles).

## 4 DISCUSSION

To our knowledge this is the first study to compare the age-dependency and stereoselectivity of the PK of tramadol and ODM in subjects older than 75 years receiving a once daily formulation.

### *4.1 Age related differences in Pharmacokinetics*

In our study, tramadol exposure based on AUC and $C_{max}$ did not differ in the two age-groups for each T enantiomer after a single dose. Similar results were calculated for total clearance and renal clearance, with the exception of (-)-T which is statistically lower in the elderly. There was a 30% higher $V_{area}/F$ and an almost proportionally longer terminal half-life in elderly subjects for each enantiomer. A 30% decrease in renal clearance associated with a proportional increase in systemic exposure and terminal half-life was observed for ODM enantiomers. The mean total amount recovered in urine was not statistically different between age groups for tramadol or ODM nor was the metabolic clearance to ODM different.

Both tramadol and ODM demonstrated stereoselective pharmacokinetics when within age group comparisons were made. This is consistent with both *in vitro* and *in vivo* findings in the literature (16). A 1:1 ratio between (+)- to (-)- tramadol enantiomers in the tablets was assumed during PK analysis but no quantitative determination was performed. The difference between the age groups in the enantiomeric ratios for both T and ODM were not statistically significant suggesting that there is no age-related stereoselectivity.

PopPK results were similar to those obtained with the NCA analysis and did not reveal age-dependency with the exception of V/F and $t_{1/2}$ which were respectively 40 and 50%, greater in the elderly. The model adequately predicts the time-course of the absorption of tramadol from the extended-release formulation through plasma concentrations. BSV was fixed for LAG1, LAG2 and D2 but the estimates were reproducible and precision was good. The confidence intervals (CI) for V/F in the young and elderly did not overlap supporting the difference found between the age-groups in the NCA. In the study population, absorption by a first-order process typically started after 20 minutes and continued for another 32 minutes. This, in combination with the typical values for the initiation of zero order absorption (LAG2), indicates that zero-order absorption begins approximately 1 hour after administration of the tablet, and continues for a further 18 hours. These findings are consistent with the intended tablet design.

Simulation of steady-state AUC predicts no age related changes however $C_{max}$ is predicted to be higher and $t_{1/2}$, longer in the elderly. These differences are not likely to be clinically significant. Likar et al. found no age-related differences in steady-state plasma concentrations of tramadol and (+)-ODM collected at 2 time points during the dosing interval in a range of IR and slow-release doses; The study was conducted in 55 patients at an ambulatory pain clinic who were < 65 years, 65-74 years and 75 years and older (36). In a review article, Lee et al reported that in single dose oral and IV studies, mean AUC of immediate-release tramadol increased by 55% in patients older than 75 years while mean terminal half-life increased by 37% compared to young. However, these differences being not statistically significant, authors concluded that dose reduction in healthy elderly patients with normal renal and hepatic function is not necessary (5).

The fact that systemic exposure ($AUC_{0\text{-}inf}$, $C_{max}$) did not differ between both age groups does not infer that the absolute bioavailability (F) of oral tramadol was similar in both age groups, as the latter was not evaluated. This was a single dose administration and tramadol absolute bioavailability of CR formulations has been shown to vary from 67% after a single dose to 87% at steady-state (6). With the exception of exposure (AUC) and $k_{el}$, interpretation of differences in PK parameters, namely V/F and Cl/F, should be made cautiously. For the analyses in this paper we have assumed that bioavailability (F) is similar between the age groups which may not be the case. If the age-related difference was due solely to an age-related difference in F, this could result in differences between the groups for apparent Cl or Vd. For example, it would require a 30 % lower absolute bioavailability in the elderly compared to the young to account for the same

higher apparent Vd observed in the elderly; a degree of difference that high is unlikely. Conversely, it would require only a 6% higher absolute bioavailability in the elderly compared to the young to solely account for the non significant 22% decrease in apparent Cl observed in the elderly.

A difference in tramadol bioavailability is one of several potential sources for the difference in PK seen in the elderly group. Total clearance was not different between the age groups despite a non-significant trend to lower renal clearance (30% for (-)-T and 23% for (+)-T) in elderly subjects. Given the relative contribution of renal clearance (20% of total body clearance) for (+)-tramadol and the sample size of the study, age-related changes are therefore less likely to be observable as significant changes in the overall clearance. Therefore, one cannot exclude that a decrease in renal clearance could also explain our results for total body clearance.

The fact that absorption parameters (LAG1, ka, LAG2 and D2) were not found age-dependent suggests that differences in V/F may not be related to factors involved in the absorption process. Other responsible differences could include age-related changes in body composition, in drug transporters and in drug clearance through metabolism and excretion.

This study excluded CYP2D6 poor metabolisers but not extensive metabolisers and measured only ODM and no other metabolites, making it difficult to draw conclusions about the total metabolic profile. However, age-dependent differences in metabolism are thought to be mostly due to decreases in liver blood flow and liver mass rather than CYP activities (2). Since tramadol is not flow-dependent, differences in hepatic clearance would therefore be unlikely. In our study, regardless of age approximately 20% of the overall dose of tramadol was recovered in urine as tramadol and approximately 12% as ODM.

Well-recognized age-related changes in body composition, such as alterations in tissue and plasma protein binding, intracellular water content, and ratio of adipose tissue to lean muscle mass, may result in an increased volume of distribution in the elderly (19, 20). Tramadol is only 20% plasma protein bound (5, 37); age-related changes in plasma protein binding are not likely to result in changes in volume of distribution. Tramadol is a basic (pKa: 9.41) and slightly lipophilic drug (logP: 1.35). A decrease in intracellular water and increase in adipose tissue are age-dependent effects on body distribution that may offset each other. Tramadol shows a high tissue affinity, in particular for skeletal muscle that accounts for 50% of body weight (5, 37).

Since muscle mass tends to decline with age, this would result in a lower tissue distribution of tramadol, in contrast to what is observed in our elderly subjects.

It is worth mentioning that differences in elimination processes from sources such as drug transporters may also have an influence on $V_{area}/F$. A study of transepithelial transport of T and ODM enantiomers using a Caco-2 cell monolayer model found that neither T nor ODM are P-gp substrates but that proton-based efflux pumps may be involved in limiting T gastrointestinal absorption and enhancing renal excretion of T and ODM (38). Therefore, differences in hepatic transporter expression with age, specifically OCT-1 transporters, could be a potential explanation for the higher ODM exposure. Tzvetkov et al. found that, while the plasma concentration-time profile of T in healthy volunteers was independent of OCT1 genotype, overexpression of OCT1 increased ODM uptake into hepatic cells by 2.4 fold (39). Conversely, the increase in ODM uptake was diminished with OCT1 inhibitors and absent with overexpression of loss-of-function genetic variants. An age-related effect of OCT1 on ODM PK and PD cannot be excluded, although Nies et al (2009) found that OCT1 expression in hepatic cells is independent of age in humans (40).

PopPK parameters pertaining to the absorption process are in agreement with the pharmacodynamics reported for this tramadol formulation. The onset of pain relief in extended-release formulations is of interest since patients may miss doses, decide to or be instructed to take medication holidays. The percentage of dose released by a first-order process (f1) was 54% and, according to our PK model, this release should have occurred within the first hour after administration. Tramadol plasma concentrations of 100 ng/mL are suggested, by some authors, to be the minimum therapeutic level while others suggest that higher concentrations are needed (37, 41). In an exploratory study of 47 patients with low back pain, plasma concentrations at the onset of perceptible pain relief were collected after administration of the same tramadol formulation examined here. Onset of analgesia occurred within 1 hour at which time mean plasma concentrations were 56 ± 38 ng/mL, supporting minimum therapeutic levels of 50-100 ng/mL.

### *4.2* Food-effect related to formulation

Five (5) individuals demonstrated a notably more rapid absorption (Figure 4) with disproportionate peak concentrations occurring 4-8 hours post-dose. These differences in PK

profile resulted in a bimodal distribution for the D2 parameter and a shorter D2 for the overall population. In these subjects, the more rapid absorption and higher peak concentrations are associated temporally with the first meal consumed after the overnight fast and, therefore, may reflect a food-effect. Higher gastric retention times occur for prolonged release tablets, particularly in the presence of high fat meals. Food-effect studies in healthy young volunteers have demonstrated that co-administration of tramadol ER 200 mg with a high fat meal resulted in a 54% increase in mean $C_{max}$ but no increase in AUC.

Attempts to include an effect of food on the formulation as a parameter in the model or as a covariate on each of the parameters resulted in an increase in OFV or a failure of the model to converge. Since the main objective of this analysis was to understand the PK of tramadol in elderly subjects and since there was a large age-related discrepancy in the number of subjects experiencing a food-effect (1 elderly versus 4 young subjects), the authors considered it more conservative to use the analysis excluding the food-effect subpopulation.

### *4.3 Adverse effects and clinical relevance*

As expected, there was a higher incidence of adverse events in both groups during active treatment as compared to placebo treatment. The percentage of adverse events was higher in younger subjects than in elderly subjects, however, due to the limited number of subjects and the limited availability of reported data on the incidence of adverse events in elderly patients (as opposed to volunteers), it is not appropriate to draw conclusions about the safety of tramadol, in real world use, from these data.

The findings of our analyses contribute greater in-depth knowledge about the disposition of tramadol, after administration of an extended–release formulation, by constructing a population model based on extensive sampling in relatively healthy elderly subjects with comparison to young healthy subjects. Data and findings of their analysis strengthen the evidence provided by Lee et al., that there are differences in the half-life of Tramadol in elderly versus young subjects after single dose administration and that initially extending dose interval might be considered in the elderly.

Other authors have noted relevant clinical differences in efficacy and safety in elderly patients and recommended not using tramadol in high-risk post-operative elderly patients, or reducing the dose (21), or using tramadol-acetaminophen combinations along with opioid antagonists as

opioid-sparing agents (42). Further work is required to determine the clinical significance of these differences in patients who have greater renal and/or hepatic insufficiency than the elderly subjects exposed in this study.

Since ODM contributes to the pharmacological action of tramadol, the clinical significance of a 35% increase in exposure and the slower elimination should be taken into consideration, especially after multiple dosing. A theoretical outcome of the higher exposure to (+)-ODM could be increased analgesic effect due to the higher affinity of (+)-ODM for the mu-opioid receptors; this could also result in greater incidence and/or severity of mu-receptor related adverse events in the elderly (43).

***5. Study limitations***.

A potential limitation of the study design and analysis is that elderly subjects had normal hepatic function according to the Child-Pugh criteria. Therefore, we were not fully able to investigate the effect of mild hepatic insufficiency on the PK following administration of once daily tramadol. Another potential limitation is the lack of intravenous data to document absolute bioavailability in the elderly. Finally, the number of patients included in each group in the study was small for use with a popPK analysis.

## 5. Conclusions

Differences in tramadol pharmacokinetics between relatively healthy elderly volunteers and young healthy volunteers are not remarkable. However, exposure to ODM and its elimination is significantly different. This is important since ODM+ is posited to be primarily responsible for the opioid analgesic effect and opioid side effects associated with tramadol administration. Chronic use of tramadol in elderly populations and particularly in the more frail elderly should carefully consider the patient's renal and hepatic function and the increased potential for opioid related side effects.

***Acknowledgements:*** The study was contracted by Labopharm Inc. MDS Pharma conducted the clinical portions of the study. Dr. Kenneth Swart of Farmovs-Parexel, Bloemfontein, Orange Free State, South Africa led the quantitative bioanalysis. The authors performed the Population PK analysis independently. Dr. Jun Li is acknowledged for his early contribution to model development and preliminary data analysis.

***Compliance with Ethical Standards***

***Conflict of Interest:*** Sybil Skinner-Robertson and Caroline Fradette were employees of Labopharm Inc. prior to Nov 2011. Sylvie Bouchard, France Varin and Mohamad Samer Mouksassi have no conflicts of interest.

***Funding:*** Labopharm sponsored the conduct of the study. The analyses presented herein were conducted independently as part of the doctoral research of Sybil Skinner Robertson.

***Ethical approval & informed consent:*** Before initiation of the study, the protocol and informed consent for this study were reviewed and approved by two independent ethics committees (Comité d'Ethique de la Recherche des Sciences de la Santé, Université de Montréal; and Investigational Review Board, MDS Pharma Services, Montreal). All subjects provided their written informed consent prior to the initiation of any study-related procedures. The study was conducted in accordance with the Declaration of Helsinki as well as the Enoncé de politique des trois Conseils. The study is registered at clinicaltrials.gov (NCT02329561).

***Author Contributions:***

Sybil Skinner-Robertson: This author made substantial contribution to study conception and design, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content; and final approval of the version to be published.

Caroline Fradette: This author contributed to study conception and design, acquisition of data, manuscript revision and final approval of the version to be published.

Sylvie Bouchard: This author contributed to study conception and design, manuscript revision and final approval of the version to be published.

Mohamed Samer Mouksassi: This author contributed to population pharmacokinetic model development, critical review of the manuscript and final approval of the version to be published

France Varin: This author made substantial contribution to study conception and design, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content; and final approval of the version to be published.

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**Tables**

**Table 1** Baseline Demographics

| | Young (18-40 years) n=20 | Elderly (≥ 75 years) n=15 |
|---|---|---|
| Age (years) | | |
| Mean ± SD | 29 ± 6.8 | 77±1.6 |
| Median | 28 | 77 |
| Range | 21-40 | 75-80 |
| Gender n (%)[1] | | |
| Male | 16 (80) | 11 (73) |
| Female | 4 (20) | 4 (27) |
| Weight (kg) | | |
| Mean ± SD | 74 ± 9.2 | 78 ± 7.4 |
| Median | 72 | 77 |
| Range | (59-98) | (65-93) |
| BMI (kg/m2) [2] | | |
| Mean ± SD | 25 ± 2.0 | 28 ± 2.6 |
| Median | 25 | 28 |
| Range | 21-28 | 25-35 |
| GFR (mL/min/1.73m2) [3] | | |
| Mean ± SD | 102 ± 16 | 67 ±1 2 |
| Median | 103 | 67 |
| Range | 76-135 | 50-90 |

BMI – Body Mass Index; GFR – Glomerular Filtration Rate , SD- standard deviation;
[a] Percentage of subjects who are male or female within the age group
[b] The difference in BMI between the age-groups was statistically significant ($p<0.001$)
[c] GFR was calculated using serum creatinine according to the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula. The difference between the age-groups was statistically significant ($p<0.001$)

**Table 2** Most commonly reported adverse events[a] by age group and active or placebo treatment

| Adverse event [b] | Young n=20 | | Elderly n=15 | |
|---|---|---|---|---|
| | Active | Placebo | Active | Placebo |
| Nausea | 9 (45) | 0 (0.0) | 2 (10) | 1 (7.1) |
| Dizziness | 7 (35) | 0 (0.0) | 3 (15) | 1 (7.1) |
| Vomiting | 5 (25) | 0 (0.0) | 3 (15) | 0 (0.0) |
| Somnolence | 2 (10) | 0 (0.0) | 2 (10) | 0 (0.0) |

[a] Adverse events reported by 10% or more of patients
[b] Number and percentage of subjects experiencing the adverse event at least once

**Table 3** Mean pharmacokinetic parameters for tramadol following single-dose administration of tramadol 200 mg extended-release tablets in young and elderly healthy subjects

| PK Parameter | (+)-tramadol | | | (–)-tramadol | | |
|---|---|---|---|---|---|---|
| | Young 18-40 years n = 20 (CV%) | Elderly ≥ 75 years n = 14 (CV%) | Percent difference relative to the young (90% CI)[b] | Young 18-40 years n = 20 (CV%) | Elderly ≥ 75 years n = 14 (CV%) | Percent difference relative to the young (90% CI)[b] |
| $AUC_{0-48}$ (ng·h/mL)[a] | 3037 (29) | 2956 (35) | 97 (77-120) | 2591** (23) | 2491** (35) | 96 (79-114) |
| $AUC_{0-inf}$ (ng·h/mL)[a] | 2764 (30) | 3003 (38) | 108 (89-132) | 2260 (24) | 2496 (37) | 110 (92-132) |
| $C_{max}$ (ng/mL)[a] | 156 (38) | 131 (51) | 87 (69-109) | 136 (34) | 114 (54) | 87 (70-108) |
| $t_{max}$ (h)[c] | 6.6 (30) | 7.1 (36) | - | 6.7 (31) | 7.5 (37) | - |
| $k_{el}$ (1/h) | 0.11 (16) | 0.080 (25) | 72* (63-81) | 0.12** (14) | 0.080** (24) | 71* (63-80) |
| CL/F (L/h/kg) | 0.47 (28) | 0.44 (35) | 94 (77-113) | 0.57** (24) | 0.53** (35) | 93 (77-111) |
| $V_{area}$/F (L/kg) | 4.4 (25) | 5.9 (37) | 134* (110-162) | 4.9** (26) | 6.6** (37) | 134* (109-162) |
| $Ae_{0-48}$ (mg) | 18 (27) | 16 (33) | 91 (73-108) | 15** (31) | 14** (32) | 90 (71-108) |
| $CLr_{0-48}$ (L/h/kg)[d] | 0.090 (37) | 0.070 (20) | 77 (66-93) | 0.10 (36) | 0.07 (20) | 70* (60-82) |

$Ae_{-0-48}$ amount excreted in urine in 48 h, CI confidence interval, CL/F- relative clearance, $CLr_{-0-48}$ renal clearance over, Cmax maximum plasma concentration, tmax - time to maximum plasma concentration, Varea/F - relative volume of distribution, Vss - volume of distribution at steady state, λz – terminal elimination rate constant

* Difference between age groups was statistically significant ($P < 0.05$)

** Difference between the enantiomers within age group was statistically significant ($P < 0.05$)

[a]Geometric mean all others are arithmetic means except tmax

[b] Mean ratio expressed as a percentage: 90% CI of the ratio of the means

[c] Median - failed normality test - Wilcoxon Signed Rank Test performed

[d] Renal clearance calculated from $AUC_{-0-48}$ and $Ae_{-0-48}$

**Table 4** Mean pharmacokinetic parameters for ODM following single-dose administration of tramadol 200 mg extended-release tablets in young and elderly healthy subjects

| PK Parameter | (+)-ODM | | | (–)-ODM | | |
|---|---|---|---|---|---|---|
| | Young (18-40 years) n = 20 (CV %) | Elderly (≥ 75 years) n = 14 (CV %) | Percent difference relative to the young (90% CI)[a] | Young (18-40 years) n = 20 (CV %) | Elderly (≥ 75 years) n = 14 (CV %) | Percent difference relative to the young (90% CI)[a] |
| $AUC_{0-48}$[a] (ng·h/mL) | 690 (28) | 785 (24) | 127 (108-151) | 703 (15) | 806 (19) | 126* (110-144) |
| $AUC_{0-inf}$[a] (ng·h/mL) | 639 (28) | 859 (24) | 136* (107-172) | 652 (19) | 873 (18) | 134* (118-152) |
| $C_{max}$[a] (ng/mL) | 29 (33) | 31 (27) | 113 (86-149) | 32 (29) | 35 (19) | 113 (99-129) |
| $t_{max}$ (h)[b] | 8.0 (46) | 11 (56) | — | 9.0 (51) | 7.0 (66) | — |
| $k_{el}$ (1/h) | 0.097 (22) | 0.067 (29) | 69* (57-84) | 0.11 ** (16) | 0.07 (27) | 64* (55-76) |
| $Ae_{0-48}$ (mg) | 12 (40) | 9.5 (23) | 82 (61-102) | 8.21 ** (35) | 7.40 ** (16) | 90 (72-107) |
| $CLr_{0-48}$ (L//h/kg)[c] | 0.23 (26) | 0.16 (18) | 70* (59-83) | 0.18 ** (27) | 0.12 ** (19) | 67* (54-100) |

$Ae_{0-48}$ amount excreted in urine in 48 h, $AUC_{0-inf}$ area under the curve from 0 to infinity, $AUC_{0-48}$ area under the curve from 0 to 48 h, $CLr_{0-48}$ - renal clearance over 48 h, $C_{max}$ maximum plasma concentration, $K_{el}$ terminal elimination rate constant $t_{max}$ time to maximum plasma concentration,.

* Difference between age groups was statistically significant ($P < 0.05$)

**Difference between the enantiomers within age group was statistically significant ($P < 0.05$)

[a]Geometric mean all others are arithmetic means except tmax,

[b] Median failed normality test - Wilcoxon Rank Test performed

[c] Renal clearance calculated from $AUC_{0-48}$ and $Ae_{0-48}$

**Table 5** Structural and covariate model exploration and comparison of objective function value during model development for tramadol 200 mg extended-release tablets using the pharmacokinetic data obtained from all subjects (n=34)

| Run | Changes to Models with MOA | Method | Convergence | Number of Parameters | Objective Function (Change) |
|---|---|---|---|---|---|
| Base Model | One compartment, BSV fixed for all parameters except CL/F and V/F | FOCE-I | Y | 14 | 3714 |
| Intermediate Models | Two compartment BSV fixed for Q, VP, $k_a$, D2, LAG1, f1 and LAG2 | FOCE-I | Y | 18 | 2391 |
| | One compartment; $k_a$ and f1 not fixed; BSV fixed for D2, LAG1 and LAG2 | FOCE-I | Y | 14 | 2310 |
| | One compartment; f1 fixed at 25%; BSV fixed for D2, LAG1 and LAG2 | FOCE-I | Y | 12 | 2317 |
| Final Model | One compartment; $k_a$ and f1 not fixed; BSV fixed for D2, LAG1 and LAG2 apparent volume with age as influencing covariate; | FOCE-I | Y | 15 | 2292 |

BSV between subject variability,CL/F apparent clearance, D2 duration of zero-order input, FOCE–I first-order conditional estimation with interaction, f1 fraction of the dose absorbed by a first-order process(immediate release from formulation), $k_a$ first-order absorption rate constant, LAG1 - lag time after which first-order absorption starts, LAG2 lag time after which zero-order absorption takes place, MOA mixed-order absorption, OFV – objective function value ,V/F apparent volume of distribution; Q rate of clearance to second compartment; VP peripheral volume, Y yes

**Table 6** Final population parameter estimates[a] of the pharmacokinetic data for tramadol extended-release 200 mg tablets in Young and Elderly subjects[b]

| Parameter (n=29) | Final Model (% RSE) | Median | RSE % based on bootstrap[c] SE | Percent difference between the median and final model | 95% CI based on the bootstrap[c] |
|---|---|---|---|---|---|
| CL/F (L/h/kg) | 0.48 | 0.48 | 5.79 | 0.02 | 0.41-0.53 |
| V/F (L/kg)) | | | | | |
| Young (n = 16) | 4.1 | 4.0 | 6.1 | -1.7 | 3.6-4.6 |
| Elderly (n = 13) | 5.5 | 5.4 | 9.1 | -1.5 | 4.6-6.5 |
| CL/F on V/F | 0.59 | 0.61 | 20 | 1.8 | 0.34-0.80 |
| $k_a$ (h-1) | 0.40 | 0.41 | 15 | 2.7 | 0.30-0.53 |
| D2 (h) | 18 | 17 | 10 | -1.8 | 16-22 |
| LAG1 (h) | 0.33 | 0.34 | 7.9 | 1.7 | 0.28-0.38 |
| f1 | 0.52 | 0.52 | 15 | 0.34 | 0.39-0.67 |
| LAG2 (h) | 0.54 | 0.54 | 38 | 0.010 | 0.50-1.4 |
| BSV CL (%) | 28 | 27 | 12 | - 4.2 | 20-33 |
| BSV V/F (%) | 0.080 | 0.07 | 24 | -8.3 | 0.04-0.11 |
| BSV $K_a$ (%) | 42 | 41 | 18 | -0.67 | 27-56 |
| BSV f1 (%) | 27 | 24 | 39 | -10 | 0.27-34 |
| ERRCV[d] (%) | 19 | 19 | 6.9 | -1.0 | 17-22 |

BSV between-subject variability expressed as %, which is computed by taking the square root of the variance of a population PK parameter that is modeled as log normal, CI confidence interval, CL/F apparent clearance,, D2 – duration of zero-order input, f1 fraction of the dose absorbed by a first-order process, $k_a$ – first-order absorption rate constant, LAG1 lag time after which first-order absorption starts, LAG2 lag time after which zero-order absorption takes place, RSE relative standard error computed as 100 x standard error/estimate, V/F apparent volume of distribution

[a] Final model was a one compartment with a dual absorption process and age as significant covariate on apparent volume.

[b] POPPK results are from analysis after exclusion of food-effect subpopulation PK data.

[c] Calculated after a nonparametric resampling bootstrap analysis stratified by age group, a total of 1000 replicates were performed

[d] Difference between the final model estimate and the bootstrap median.

[e] proportional error in percent

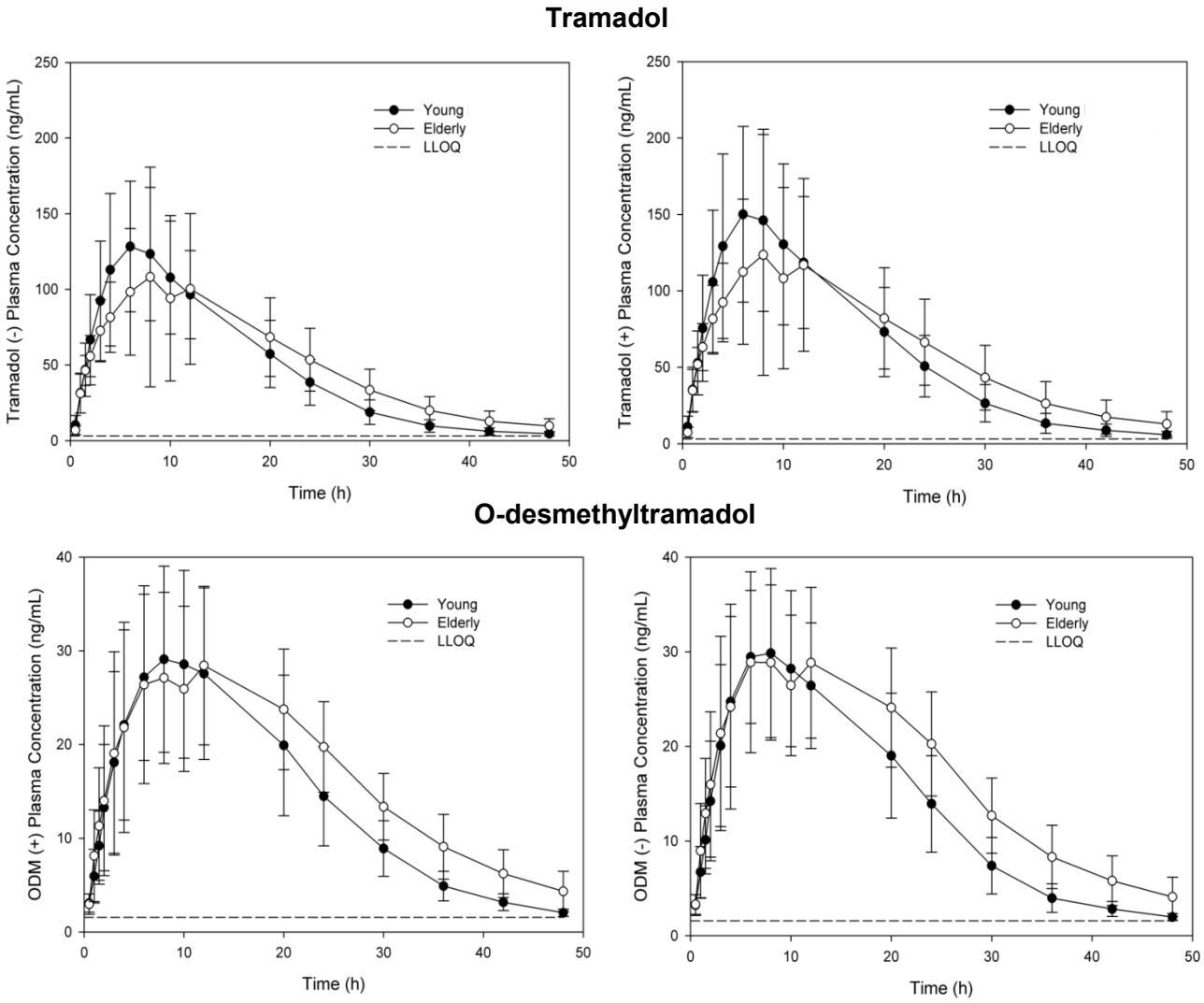


**Fig.1 Mean (±standard deviation) plasma concentrations of tramadol and O-Demethyltramadol enantiomers over time after single-dose oral administration of tramadol extended-release 200 mg tablets in young and elderly subjects**

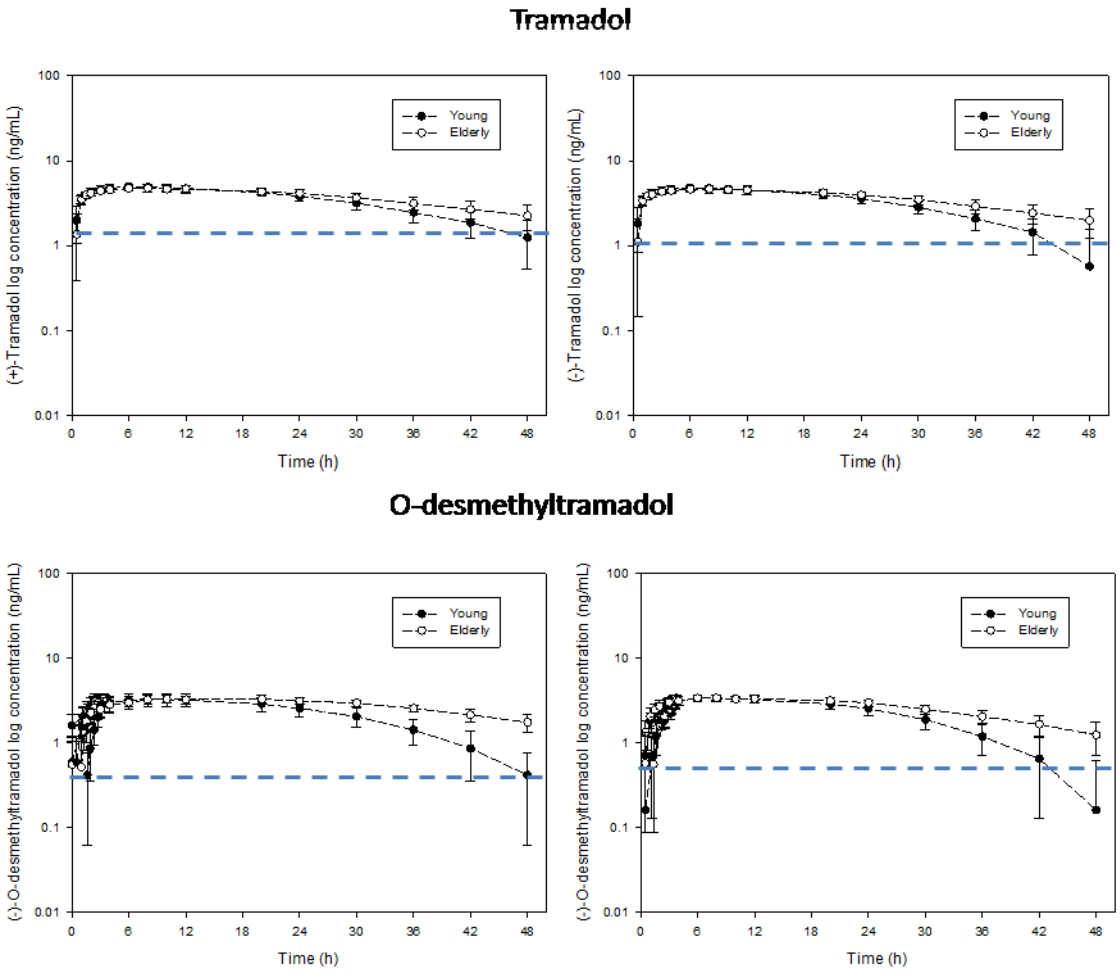


**Fig..2 Mean (±standard deviation) log plasma concentrations of tramadol and O-Demethyltramadol enantiomers over time after single-dose oral administration of tramadol extended-release 200 mg tablets in young and elderly subjects**

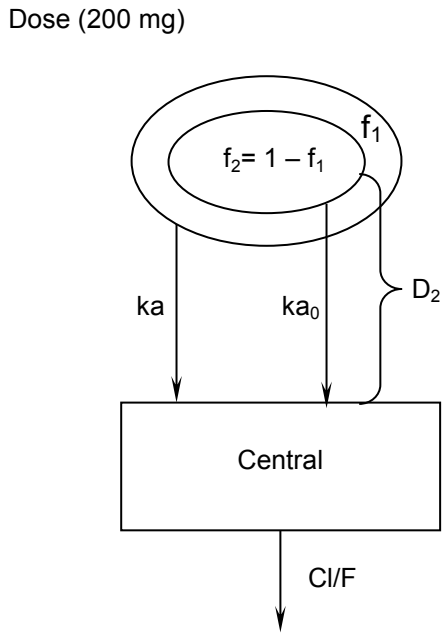


**Fig. 3 Final Structural Model for tramadol extended release 200mg**

$f_1$ fraction of the dose with first-order release, $f_2$ fraction of the dose with zero-order release ($f_2 = 1- f_1$), ka absorption rate constant of the first-order release portion of the tablet, $ka_0$ absorption rate constant of the zero-order release portion of the tablet, D2 duration of zero-

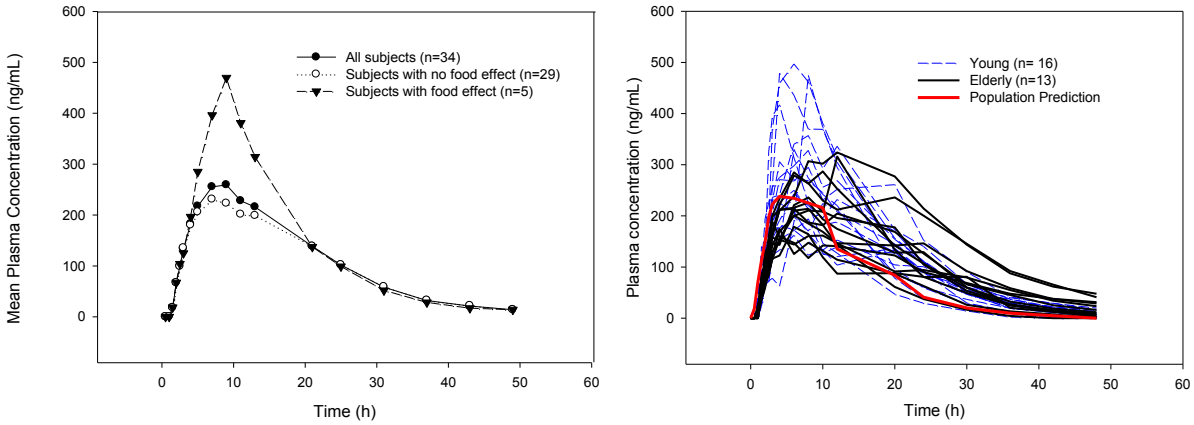


**Fig. 4 Tramadol plasma concentration-time profiles**

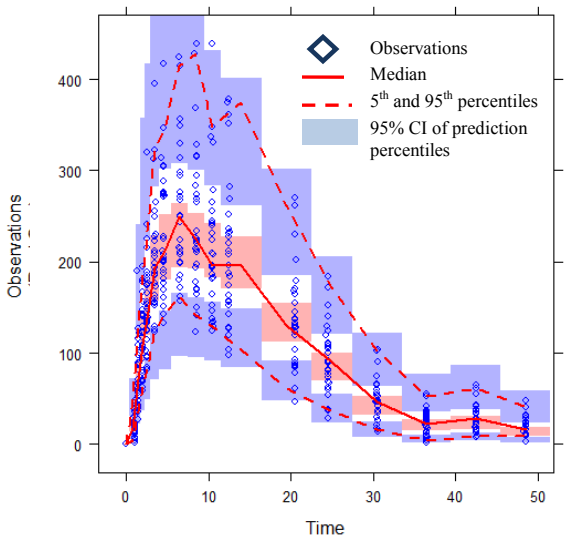


Fig. 5 Visual predictive check plot illustrating the simulation-based prediction interval overlaid on (prediction corrected) observations vs. time (h) (Run 0)

# Chapter 5: Evaluation of an experimental pain model by noncompartmental analysis (Manuscript 2 –Submitted to Pain Physician).

## 5.1 Introduction

This second manuscript presents the results of exploratory analyses of the PD data that we conducted to assess differences between young and elderly subjects with regard to pain tolerance of transcutaneous electrical stimuli at 250 Hz and 5 Hz for our ESPM. We wanted to determine whether the ESPM utilised in the study is able to detect a difference in elderly and young subjects at 5 Hz and 250 Hz after a single dose of placebo and tramadol. Furthermore, it allowed us to select the the most reliable frequency for the (+)-ODM PK PD analysis presented in the third paper. This work laid the basis to select the frequency of stimulation in the ESPM and to characterise the effect curve to assist in developing the (+)-ODM PK/PD model.

Sybil Skinner-Robertson made substantial contribution to study conception and design, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content; and final approval of the version to be published. Her contribution to the writing of the manuscript is estimated at 80%, Dr. Varin and Dr. Mouksassi having made an estimated contribution of 20%.

## 5.2 Manuscript

**Evaluation of an experimental pain model by noncompartmental analysis.**

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**ABSTRACT:**

**Background:** Understanding analgesic pharmacodynamics (PD) in the elderly is key to optimising pain management. Electrically stimulated pain models (ESPM) permit assessment of pain responses in humans. C and Aδ sensory fibres convey pain and respond to low frequency electrical stimulus (5 and 250 Hz, respectively). Human research suggests pain tolerance threshold (PTT) is similar or decreases with age.

**Objectives:** To determine whether an ESPM is able to detect a difference in PTT in elderly (≥75 years) and young (20-40 years) subjects after single dose administration of a placebo and tramadol, a low potency analgesic.

**Study design:** Two-cohort, randomised, placebo-controlled, cross-over study

**Methods:** A noncompartmental analysis of data at 17 timepoints on 5 Hz and 250 Hz PTT over 24 h.

**Results:** Young (16) and elderly (13) subjects showed similar baseline (E0) PTT between active and placebo both overall and by age group in both frequencies. Net drug effect took into account negative and positive changes from E0. In the elderly, net peak effect on PTT produced by active was significantly greater for both 5 Hz (34%) and 250 Hz (30%). Net area under the 24-h effect-time curve during active treatment was significantly higher for both 5 Hz (163 %) and 250 Hz (175%) stimulations in the elderly. No clinically significant difference was observed in the young.

**Limitations:** High variability in young subjects, despite efforts to remove outliers limited our ability to draw conclusions in that age group. Generalizability of results obtained from an experimental pain model in volunteers to treatment of elderly patients may be limited.

**Conclusion:** ESPM can detect a difference for pain tolerance threshold between placebo and tramadol administration in the elderly. Although both 5 Hz and 250 Hz stimulations can detect a difference, the effect size for 5 Hz is larger and seems more precise and reliable, particularly in the elderly.

## 1 INTRODUCTION

Pain is a complex sensory, affective and cognitive experience. Determination of analgesic efficacy in humans using animal models only gives part of the picture while results from clinical trials are affected by concomitant medications and coexisting morbidities. Human experimental pain models offer the opportunity to assess human responses to pain in a more controlled setting using objective measures. Electrically Stimulated Pain Models (ESPMs) can selectively activate different afferents and nervous structures and thereby evoke various pain sensations (1). The reliability of ESPM to detect differences in current perception threshold has recently been established for potent post-operative analgesia (2). However, differences in pain tolerance threshold (PTT) have not been established for a low potency analgesic such as tramadol and not in an elderly study population.

With age peripheral nerves display structural, functional and biochemical changes that primarily affect Aδ and C-fibres. Electrical current stimulation predominantly stimulates C, Aδ and Aβ fibres (3). C and Aδ fibres are high threshold afferents which convey pain and temperature sensations (4) and which respond to low frequency electrical stimulus (e.g. 5 and 250 Hz, respectively) after several milliseconds of continuous depolarization. Previous work has demonstrated the utility of an ESPM at 5 Hz in determining sensory blockade with ropivacaine, a potent local anesthetic, before and after orthopedic surgery (5, 6). Furthermore, ESPMs have been used to study analgesic response in a variety of strong opioids including morphine, alfentanil and remifentanil (7-10).

Tramadol is a centrally acting analgesic which demonstrates weak opioid action and modifies descending pain transmission through inhibition of monamine reuptake. Its analgesic potency is comparable to codeine and dextropropoxyphene (11, 12). Although optimising pain management in the elderly requires a systematic understanding of the pharmacodynamics (PD) of analgesics in the elderly, few studies have been conducted to assess the efficacy and safety of analgesics in this population (13, 14). PKs have been studied but a quantitative tool that would allow PK/PD studies of analgesics vs subjective assessment is needed. Data from a study utilising an ESPM to assess differences between young and elderly subjects with regard to pain tolerance of transcutaneous electrical stimuli at 250 Hz and 5 Hz are presented here. The objective of these exploratory analyses is to examine whether the ESPM utilised in the study is able to detect a difference in elderly and young subjects at 5 Hz and 250 Hz after a single dose of placebo and tramadol.

## 2 MATERIALS AND METHODS

### 2.1 Experimental design

Drug effect data from a study conducted between January and February 2007 that was intended to evaluate the PK and PD after a single dose of Tramadol Contramid® ER tablets in elderly (≥ 75 years) and healthy young (18-40 years) volunteers are analysed and presented here. This two-cohort, randomised, placebo-controlled, cross-over, study used an ESPM to evaluate PTT. Subjects received either a single oral dose of 200 mg Tramadol Contramid® OAD controlled-release tablets or identical placebo with a 7-day washout between each period. The study was conducted at a phase 1 facility (MDS Pharma Services, Montreal, Quebec) where subjects were confined for 12 h prior to dosing and for 48 h afterwards. The sequence of administration was randomized and double blinded. Each subject was assigned a unique identification number and received the corresponding product according to a randomization scheme taking into account age to ensure an equal number of young and elderly subjects in each treatment sequence.

Noncompartmental (NCA) and population PK analyses were reported in an earlier publication (15). Data from this study is used here to assess the ability of 5 Hz and 250 Hz transcutaneous electrical stimuli to detect a difference in PTT response between placebo and active treatment in

young and elderly subjects. A future publication, will present a PK/PD analysis of 0-desmethyltramadol, tramadol's active metabolite, in young versus elderly subjects(16).

Before initiation of the study, the protocol and informed consent for this study were reviewed and approved by two independent ethics committees (Comité d'Ethique de la Recherche des Sciences de la Santé, Université de Montréal; and Investigational Review Board, MDS Pharma Services, Montreal). All subjects provided their written informed consent prior to the initiation of any study-related procedures. The study was conducted in accordance with the Declaration of Helsinki as well as the Enoncé de politique des trois Conseils. The study is registered at clinicaltrials.gov (NCT02329561).

**2.2 Subjects**

At screening, subjects were determined to be healthy based on medical history, physical examination, and evaluation of vital signs, electrocardiogram (ECG), and clinical laboratory data. Subjects with an increased risk of seizures or conditions that would affect sensory nerve conduction were excluded; as were subjects with bowel disease affecting absorption or previous failure of treatment with tramadol or discontinuation of treatment due to adverse events. Female subjects of childbearing potential had to have negative pregnancy test results at screening and clinic check-in for each study period. Use of all medication (including over-the-counter products) was prohibited for 7 days prior to dosing and during the time of sample collection with two exceptions: elderly subjects were permitted to continue taking stable doses of chronic medications, other than strong CYP inhibitors/inducers, and female subjects were permitted to continue taking hormonal contraception or replacement therapy. Use of any non-excluded concomitant medications was recorded.

**2.2 Pharmacodynamic evaluations**

PD data were collected using the Neurometer® CPT/C (Neurotron, Inc., Baltimore, MD, USA), a fully automated quantitative neuro-diagnostic device that generates constant alternating current sinusoid waveform stimuli at 3 different calibrated frequencies (2000 Hz, 250 Hz and 5 Hz). The device has a possible range from 0.01 milliAmperes (mA) to 10 mA (with an automatic cut-off at 10mA) (17-19). The Neurometer® was used to measure PTT which was defined as the maximum

amount in mA of the atraumatic neuroselective electrical stimulus that a volunteer was willing to tolerate. We utilised the 250 Hz and 5 Hz stimulus to selectively target, respectively, Aδ and C fibres which convey pain and temperature sensations (4). We did not use the 2000 Hz frequency which stimulates fibres that convey information about touch and pressure since we are testing a pain model (4).

Prior to administering tramadol, we ensured that the subjects were familiar with the electrical stimulus procedure, sensations they might experience and how to stop the test if they wished to. On the evening prior to their first dose, subjects received training during which they had at least two practice procedures.

In order to administer the painful stimulus, two 1-cm diameter gold-plated surface electrodes linked to the Neurometer® were applied to the non-dominant middle finger of each subject during data collection sessions. If cuts, scrapes, contusions, healing wounds or other signs of recent trauma were present on the non-dominant middle finger, the dominant middle finger or non-dominant index finger were used. Electrical stimulations were conducted at the following times: prior to dosing and at 0.33, 0.75, 1.25, 1.75, 2.5, 3.5, 4.5, 5.5, 7, 9, 11, 14, 20, 24 and 30 hours after dosing. Stimulations occurred at least five minutes apart and at each time point, the 250 Hz stimulation was applied first. Since the study also collected PK data, the ESPM ratings were conducted prior to PK sampling to avoid influencing the subjects' pain tolerance. Subjects were isolated from each other by means of cardboard dividers during data collection periods; noise and other stimuli were kept to a minimum and subjects were asked to remain sitting and minimise physical activity during the first 4 hours after administration of tramadol.

**2.3 Data**

All recorded data from the PD evaluations were entered into Microsoft Excel 2010® (Microsoft Corporation, Redmond, WA) and double verified for accuracy. Initial cleaning of the database to remove duplicates and obvious outliers (20) as well as initial establishment of baseline was conducted prior to un-blinding of the data. Initially we intended to utilise the value recorded at Time 0 ($t_0$) for baseline. However, visual inspection of the data demonstrated large variability in PTT for both 5 Hz and 250 Hz in the early sampling times and after 24 hours. Therefore, baseline for each period was estimated from the values at $t_0$ and the last recorded value (21). Data after 24 hours were not used for the noncompartmental analysis to ensure that measurable

tramadol concentrations would be observed in all subjects in the active period thus providing a meaningful comparison with the placebo period.

## 2.4 Analyses

### 2.4.1 Demographic analysis

Descriptive statistics including mean, median, standard deviation (SD) and range were calculated for demographic variables using Sigmaplot® 11.0 (Systat Software, San Jose, CA).

### 2.4.2 Pharmacodynamic analysis

A noncompartmental analysis was conducted to describe the PTT in young and elderly subjects during placebo and active administration phases using model 220 of Phoenix® WinNonlin® version 6.4 software (Certara USA, Inc., Princeton, NJ). The dependent variable, PTT after 5 Hz and 250 Hz stimulations, were provided at time of observation as well as at dosing time. Determination of baseline response ($E_0$) was carried out as described above for each administration phase (active or placebo). For each subject and administration phase (active or placebo), individual area under the effect-time curve (AUEC) between 0 and 24 h was calculated using the linear trapezoidal rule. Both positive and negative fluctuations from the predetermined baseline response were taken into account during integration and calculated as $AUEC_{above}$ and $AUEC_{below}$, respectively. Summation of all positive and negative AUEC yielded $AUEC_{net}$. Maximum effect ($E_{max}$), Time to maximum effect ($T_{max}$), Time above baseline ($T_{above}$), and Percentage change from $E_0$ to $E_{max}$ ($\Delta E_{max}$ (%)) were also analysed.

A linear mixed effect regression model (LMEM) (Phoenix® WinNonlin® version 6.4) was utilised to compare the results amongst the age and administration phases to determine whether the ESPM at each stimulus frequency was able to detect a difference between placebo and active administration phases and between those administration phases in young and elderly subjects. Least squares means (LSM) point estimates for each parameter and for the difference between the parameters overall, by age and by administration phase were calculated along with standard error of the means, 95% confidence intervals (CI) and p-values (significant < 0.05). To compare our data with the literature on placebo effect, Cohen's d for $E_{max}$ was calculated as follows:

(mean $E_{max}$ for active (A) - mean $E_{max}$ for placebo (P)) / Standard Deviation (SD) for pooled; SD pooled was calculated as $\sqrt{(SD_A+SD_P)/2}$ (22).

## 3 RESULTS

### 3.1 Demographics

A total of 20 young and 15 elderly subjects were enrolled in the study. One subject from the elderly group discontinued early in the first period due to personal reasons and was excluded from the analyses. Five subjects, 4 from the young group and 1 from the elderly group, were excluded from analyses due to a food effect as described in detail in Skinner Robertson et al.'s previous report (15). The analyses presented here included 29 healthy young and elderly subjects (Table 1) most of whom were male. In the first cohort of patients, a concealed electrical panel at the research clinic interfered with the functioning of one of the neurostimulation devices by spontaneously shutting it down at times before PTT was reached and thereby delaying data acquisition (less than 10 min). The issue was resolved by the time the second cohort was brought to the clinic for testing. Despite this, there was no statistically significant cohort effect.

### 3.2 *Comparison of active and placebo period in patients regardless of age group*

Table 3 presents the data observed for effect at $E_0$ and $E_{max}$ and $\Delta$ $E_{max}$ (%). The data are presented for all patients and by age group for active and placebo as the LSM point estimate (mean) and difference of the means with the 95% confidence interval. All point estimates and all differences in the means were within the 95% CI.

Adverse events reported by at least 10% of subjects are presented in Table 2.

Both when all patients were considered and when the age groups were compared, there were no differences by administration phase (placebo versus active) at baseline ($E_0$) for PTT under 5 Hz or 250 Hz stimulation.

Maximum effect and $\Delta$ $E_{max}$ (%) were significantly greater in the active versus placebo administration phases for both 5 Hz and 250 Hz stimulations when patients were compared

regardless of age group (Table 3).

The results of the noncompartmental analysis of the data by treatment regardless of age group are presented as Whisker plots in Figure 1. For both 5 Hz and 250 Hz stimulations, the point estimate for the difference between active and placebo means was statistically higher for $AUEC_{above}$ after 5 Hz (511, 95% CI [152-871]; 54% relative increase) and 250 Hz (566, 95% CI [141-991]; 58% relative increase); for $AUEC_{net}$ after 5 Hz (612, 95% CI [223-1002]; 75% relative increase) and 250 Hz (625, 95% CI [183-1068]; 57% relative increase); and, $Time_{above}$ after 5 Hz (4.14 h, 95% CI [1.38-6.90 h]; 22% relative increase) and 250 Hz (3.37 h 95% CI [0.79-5.95h]; 18% relative increase). $AUEC_{below}$ was significantly lower only for stimulation with 5 Hz.

***3.3 Comparison of active and placebo phase by age group***

Mean results by stimulation frequency, administration phase and age group are presented in Table 3. All point estimates and means were within the 95% CI. The SE is lower in the 5 Hz group consistently.

For $E_0$, no differences were observed between placebo and active administration phase in the young and elderly groups under either 5 Hz or 250 Hz stimulation (Table 3). In elderly subjects, there was a significantly higher $E_{max}$ and $\Delta E_{max}$ during the active administration phase after both 5 Hz and 250 Hz stimulations while a higher $\Delta E_{max}$ (but not $E_{max}$) was observed during active administration phase in young subjects only after 250 Hz stimulation (Table 3).

Whisker plots of the results of the NCA by stimulation frequency, administration phase and age group are presented in Figure 2. For the 5 Hz stimulation, the interquartile range (IQR) was greater in young subjects, particularly during placebo administration, with the exception of $AUC_{below}$. For the 250 Hz stimulation, the IQR was greater in young subjects than elderly subjects, with the exception of $AUEC_{net}$.

In young subjects, difference in the point estimate between the means for active versus placebo administration phases were not statistically different for $AUEC_{above}$ , $AUEC_{net}$ and $AUEC_{below}$.

In elderly subjects, the point estimate for the difference between the means showed a significantly higher $AUEC_{above}$ (5 Hz: 906 mA, 95% CI [355-1457] relative difference: 118% higher) 250 Hz: 695, 95% CI [44-1347] relative difference: 116% higher or two-fold difference), and $AUEC_{net}$ (5 Hz: 1009 mA, 95% CI [412-1606] relative difference: 163 % higher or almost 3-fold difference; 250 Hz: 734 Hz, 95% CI [56-1412] relative difference: 175% higher or almost 3-fold difference) during active administration for both 5 Hz and 250 Hz stimulation. $Time_{above}$ was significantly longer only for the 5 Hz stimulation in elderly subjects (5 Hz: 5.02 h, 95% CI [0.80-9.26] relative increase: 35% higher).

## 4.0 DISCUSSION

The objective of this analysis was to determine whether the ESPM, using the 5 or 250 Hz frequency, was able to capture changes in tolerance to pain intensity using PTT after the administration of a weak opioid in healthy volunteers. During analysis, we also explored whether an age related difference in response existed between elderly and young subjects. This study demonstrated that in elderly patients an ESPM is able to detect a difference in pain tolerance between placebo and active administration phases. Although the difference can be detected for both 5 Hz and 250 Hz, the effect size for 5 Hz is larger and seems more precise and reliable particularly in the elderly.

Although currently open to debate, placebo control in clinical studies is traditionally accepted by the scientific community as the best way to determine the true effect of a medication, based on the premise that there is an underlying effect of placebo and that true medication effect is additive to that of the placebo effect (23). Placebo response is highly variable and depends on many contextual factors (22), this is particularly true in analgesic studies and therefore our study had a placebo control arm.

To ensure that the ESPM was able to detect a difference between active and placebo administration phases, we first examined the data by administration phase (placebo versus active) without taking into consideration age group and found no significant differences at baseline in PTT between the active and placebo groups with either frequency. In our study, when subjects were administered placebo the maximum value for PTT over baseline ($\Delta E_{max}$) was

increased by 81%. Vase et al., in their meta-analysis of 21 articles published between 2002 and 2007, found a highly variable magnitude of placebo analgesia with effect size calculated using Cohen's D ranging from 0.12 to 2.51. The average effect size in studies where placebo is used as a control for various conditions ranged from 0.15 to 0.27 (22, 24-26). In our study, it was 0.25 and 0.11 for the 5 Hz and 250 Hz stimuli, respectively. When comparing active administration phase versus placebo, the ESPM was able to detect a maximum relative increase from baseline of 29% and 24% for the 5 Hz and 250 Hz electrical stimulations, respectively. Similarly, $AUEC_{above}$, which is a pharmacodynamic measure of exposure (duration x amplitude of positive effect) increased by 75% for both frequencies. Thus, the ESPM was adequately able to detect a difference between placebo and active administration phases at either stimulation frequency.

There were no statistically significant differences either regardless of age or when age was taken into account when the data and analyses for the 5 HZ and 250 Hz stimulations were compared. The confidence intervals for differences in the means were consistently narrower for the 5 Hz analyses suggesting that we are able to more accurately estimate the difference in the 5 Hz data. This could be because the sensation caused by the 5 Hz stimulation is more unpleasant and therefore easier to recognize consistently.

When analyses were conducted to take into account the age related differences in pain tolerance, there were no significant differences in $E_0$ between the age groups with 5 Hz or 250 Hz stimulation. Studies in humans, in general, have drawn inconsistent conclusions with regard to the purported increase in pain perception and the decrease in pain tolerance in the elderly (27). In experimental studies the modality of the painful stimulus seems to play a key role. Pain perception has been shown to decrease with thermally induced pain (28-31) and increase with mechanically induced pain (32, 33). Results of published studies of age related changes in pain tolerance using electrical nociceptive stimuli are less clear with one demonstrating a no change (34), two demonstrating reduced pain perception. Our exploratory results for pain tolerance showed baseline PTT in elderly showing a trend to be lower than in the young.

Data in the young group failed to demonstrate significance against placebo in any of the analyses except for $\Delta E_{max}$ after 250 Hz stimulation. The clinical significance of this observation is

debatable as no difference was observed between active and placebo AUECs in young subjects. In our opinion, AUEC is a more robust indicator of the persistence of effect. The point estimates for the mean $AUEC_{above}$ and $AUEC_{net}$ were consistently higher in the elderly during active administration phase for both 5 Hz and 250 Hz stimulations. A plausible reason for the fact that only elderly subjects showed a consistent and sustained increase in PTT during the active phase was identified in our previous noncompartmental PK analysis where a 30% higher exposure to (+)-0-Desmethyltramadol (+-ODM) was observed in elderly patients (15). As this metabolite is associated with much of the opioid analgesic effect of tramadol, this would roughly correspond to the 30% higher $AUEC_{above}$ and $AUEC_{net}$ observed in the elderly compared to young during the active period.

Within the elderly age group, the analyses showed that while both the 5 Hz and 250 Hz ESPM were able to reliably detect a difference between active and placebo administration phases, variability was smaller in the 5 Hz results for the elderly. The greater reliability of the 5 Hz versus the 250 Hz frequency could be particularly relevant in the elderly age group due to changes in the detection, processing and modulation of pain signals related to age. Age related structural and functional impairment in peripheral nerves is most notable in A-δ fibres which are selectively stimulated by the 250 Hz frequency of the Neurometer® (19, 27, 35). Therefore, the 5 Hz data will form the basis for future PK/PD modeling of the data.

**Limitations**

For most measures, variability is higher in the young group with both the IQR (25% and 75%) and the $10^{th}$ and $90^{th}$ percentile error bars usually being greater. This is evident despite efforts to remove outliers during early visual inspection of the data. We speculate that the greater variability is a result of a desire of some of the younger subjects to test whether their pain tolerance would be higher than the cut-off limit of the Neurometer® apparatus. Including an older young group, such as 30-50 year olds may have reduced the attempts to test the limits of the machine and reduced variability. Since the objective of the ESPM is to demonstrate changes in pain tolerance and not the maximum tolerance of a given individual, anchoring the rating to a visual analogue scale to help the subjects more consistently determine their PTT could have further reduced variability. Also, elderly subjects are more experienced in gauging their pain tolerance.

Finally, one may also question the generalizability of the results obtained from an experimental pain model conducted in volunteers to treatment of elderly patients. However, Olesen et al. suggest that experimental pain models offer the opportunity to study pain responses when they are not blurred by other symptoms and where confounding environmental circumstances are as controlled as possible (1). Development of a population PK/PD model that links the ESPM to the concentrations of O-desmethyltramadol will be important future work to determine how age related factors affect the pain response of elderly subjects administered tramadol.

## 5.0 CONCLUSIONS

ESPM is able to detect a difference between placebo and active administration phases for pain tolerance threshold in the elderly. Although both 5 Hz and 250 Hz can detect a difference, the effect size for 5 Hz is larger and seems more precise and reliable particularly in the elderly.

## References


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**Table 1. Baseline Demographics**

| | Young (18 - 40 years) n = 16 | Elderly ( ≥ 75 years) n = 13 |
|---|---|---|
| Sex n (%)[a] | | |
| Male | 13 (81) | 10 (77) |
| Female | 3 (19) | 3 (23) |
| Weight (kg) | | |
| Mean ± SD | 74 ± 10 | 78 ± 7 |
| Range | 59 – 98 | 65 – 93 |
| BMI ($kg/m^2$)[b] | | |
| Mean ± SD | 25 ± 2 | 28 ± 3 |
| Range | 21 – 27 | 25 – 35 |
| GFR ($mL/min/1.73m^2$)[c] | | |
| Mean ± SD | 103 ± 14 | 68 ± 12 |
| Range | 78 – 135 | 50 – 90 |

SD- Standard Deviation; BMI – Body Mass Index; GFR – Glomerular Filtration Rate

[a] Percentage of subjects who are male or female within the age group

[b] The difference in BMI between the age-groups was statistically significant ($p<0.001$)

[c] GFR was calculated using serum creatinine according to the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula. The difference between the age groups was statistically significant ($p<0.001$)

**Table 2 Most commonly reported adverse events[a] by age group and active or placebo treatment (15)**

| Adverse event [b] | Young n=20 | | Elderly n=15 | |
|---|---|---|---|---|
| | Active | Placebo | Active | Placebo |
| Nausea | 9 (45) | 0 (0.0) | 2 (10) | 1 (7.1) |
| Dizziness | 7 (35) | 0 (0.0) | 3 (15) | 1 (7.1) |
| Vomiting | 5 (25) | 0 (0.0) | 3 (15) | 0 (0.0) |
| Somnolence | 2 (10) | 0 (0.0) | 2 (10) | 0 (0.0) |

[a] Adverse events reported by 10% or more of patients
[b] Number and percentage of subjects experiencing the adverse event at least once

**Table 3 Least square mean PTT and difference of means for 5 Hz and 250 Hz stimulations during active and placebo administration phases.**

| | | 5 Hz | | | 250 Hz | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **Subjects** | **Placebo LSM [95% CI]** | **Active LSM [95% CI]** | **Difference of the means [95% CI]** | **Placebo LSM [95% CI]** | **Active LSM [95% CI]** | **Difference of the means [95% CI]** |
| **$E_0$ (uA)** | All | 142 | 143 | 1.28 | 202 | 201 | -1.25 |
| | n=29 | [104-180] | [106-181] | [-14-16] | [157-247] | [156-246] | [-28-26] |
| | Young | 142 | 140 | -2.42 | 216 | 198 | -18 |
| | n=16 | [104-180] | [89-190] | [-22-17] | [156-277] | [137-258] | [-54-16] |
| | Elderly | 142 | 147 | 4.97 | 187 | 204 | 16 |
| | n=13 | [86-198] | [91-203] | [-18-28] | [137-258] | [136-271] | [-26-58] |
| **$E_{max}$ (uA)** | All | 240 | 281 | 41* | 310 | 354 | 43* |
| | n=29 | [177-304] | [217-345] | [14-67] | [229-392] | [272-436] | [3.33-84] |
| | Young | 246 | 249 | 2.51 | 343 | 348 | 4.31 |
| | n=16 | [161-331] | [164-334] | [-32-37] | [234-453] | [239-457] | [-48-56] |
| | Elderly | 235 | 313 | 79* | 277 | 360 | 83* |
| | n=13 | [140-329] | [219-408] | [37-120] | [155-399] | [239-482] | [21-145] |
| **$\Delta E_{max}$ (%)** | All | 81 | 111 | 29* | 55 | 79 | 24* |
| | n=29 | [61-101] | [91-131] | [6-53] | [39-70] | [63-94] | [12-34] |
| | Young | 77 | 84 | 7 | 63 | 80 | 17* |
| | n=16 | [51-104] | [58-110] | [-23-37] | [42-83] | [60-101] | [3-31] |
| | Elderly | 85 | 137 | 52* | 47 | 77 | 30* |
| | n=13 | [55-115] | [108-167] | [16-88] | [24-70] | [54-100] | [13-47] |

* p value < 0.05LSM: least squares mean; PTT: pain tolerance threshold; $E_0$: baseline PTT; CI: confidence interval; $E_{max}$: maximum PTT; $\Delta E_{max}$ (%): percent difference between $E_{max}$ and $E_0$ values.

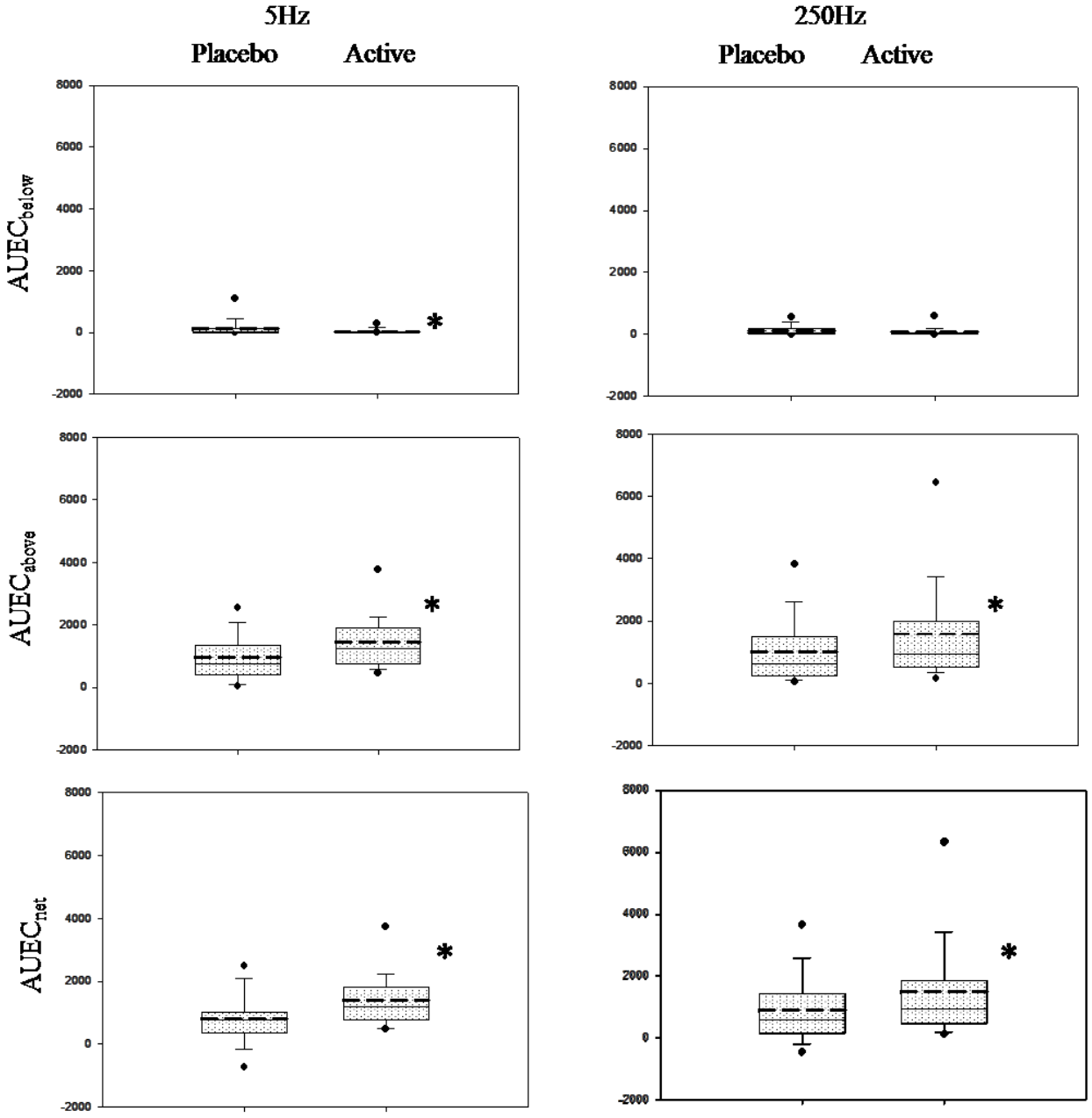


**Figure 1 Noncompartmental analysis of PTT response after 5 Hz and 250 Hz stimulations during placebo and active phases in all subjects.**

PTT: pain tolerance threshold; Hz: hertz; AUEC: area under the effect-time curve; $AUEC_{above}$: AUEC above baseline value; $AUEC_{below}$: AUEC below baseline value; $AUEC_{net}$: Difference between $AUEC_{above}$ and $AUEC_{below}$.

Note: 25th percentile: boundary of the box closest to zero; mean: dashed line within the box; median: solid line within the box; 75th percentile: boundary of the box farthest from zero; Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles.

* Difference of the means statistically significant at $p < 0.05$

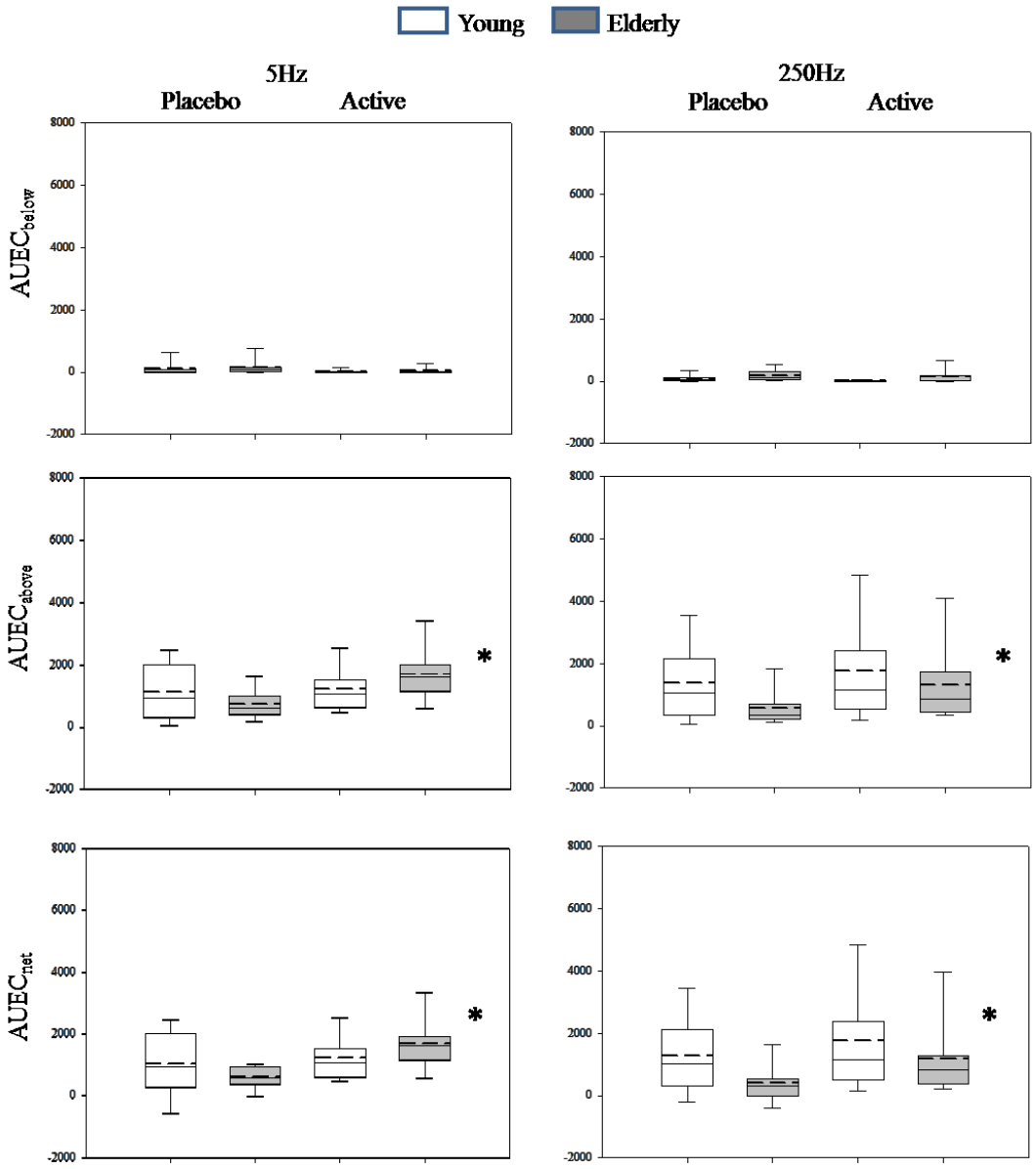


**Figure 2 Noncompartmental analysis of PTT response after 5 Hz and 250 Hz stimulations during placebo and active administration phases in young and elderly subjects.**

PTT: pain tolerance threshold; Hz: hertz; AUEC: area under the effect-time curve; $AUEC_{above}$: AUEC above baseline value; $AUEC_{below}$: AUEC below baseline value; $AUEC_{net}$: difference between $AUEC_{above}$ and $AUEC_{below}$.

Note: 25th percentile: boundary of the box closest to zero; mean: dashed line within the box; median: solid line within the box; 75th percentile: boundary of the box farthest from zero ; Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles.

* Difference of the means statistically significant at $p < 0$.

## 6. Population PK-PD modeling of O-desmethyltramadol in young and elderly healthy volunteers (Manuscript 3 – Submitted to Drugs and Aging).

## 6.1 Introduction

The overall objective of this research program is to provide information on the PK and PD of both tramadol, a weak opioid analgesic widely used in the elderly, and its active metabolite ODM in subjects 75 years and older in order to determine whether there are age related differences. Having characterised the PK of tramadol and ODM and their enantiomers and identified an appropriate ESPM and frequency to provide reliable data for the PD evaluation, our next step was to develop a PK/PD model for (+)-ODM. To our knowledge this is the first model developed to characterise PK/PD of (+)-ODM in subjects 75 and older. Our intent was to describe any age related differences in the PK/PD of (+)-ODM, the active metabolite of tramadol, using the PTT as a biomarker for analgesic effect.

Sybil Skinner-Robertson made substantial contribution to study conception and design, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content; and final approval of the version to be published. Her contribution to the writing of the manuscript is estimated at 80%, Dr. Varin and Dr. Mouksassi having made an estimated contribution of 20%.

## 6.2 Manuscript

**Population PK-PD modeling of O-desmethyltramadol in young and elderly healthy volunteers.**

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Running Title: PK/PD of O-desmethyltramadol in the elderly

**ABSTRACT**

Background: Age-related changes in concentration-effect relationship of (+)-O-desmethyl-tramadol ((+)-ODM), tramadol's active metabolite, are not documented in the elderly.

Objective: The objective of this study was to characterise, in elderly and young subjects, (+)-ODM pharmacokinetic (PK) and pharmacodynamic (PD) relationship to examine the effect of age after single dose administration of tramadol 200 mg extended-release tablets.

Methods: A population analysis of a double-blind, randomized, placebo-controlled, two-period cross-over study including 13 elderly (≥75 years) subjects with mild renal insufficiency and 16 young (18-40 years) subjects was conducted. For 48 hours post-dose, blood samples were collected and pain tolerance thresholds measured using an electrically stimulated pain model. A PK/PD model incorporating a one compartment PK model for (+)-ODM parameterized with first-order formation rate, clearance ($CL/f_m$), volume of distribution ($V/f_m$) and a sigmoid $E_{max}$ model incorporating baseline ($E_0$) and placebo effect was used.

Results: Maximum plasma concentrations of (+)-ODM occurred later and plasma concentrations declined more slowly in the elderly than in young subjects. In the elderly, $V/f_m$ was 76% larger and $CL/f_m$ 16% slower. Baseline ($E_0$) and sensitivity ($C_{50}$) for pain tolerance were similar between young and elderly subjects. However, the $E_{max}$ parameter was 2.5 times higher in the elderly and maximum possible treatment-related effect was 169 [135 - 221] in the young and 194 [149 - 252] in the elderly that is 15% higher in the elderly.

Conclusions:

This exploratory analysis suggests that age-related differences including a 76% larger distribution outside the central compartment and 16% slower clearance of (+)-ODM. These PK changes are associated with a 15% higher maximum possible treatment-related effect and carry the potential for greater efficacy but also the potential for increased side effects at the same dose in elderly subjects.

# 1 INTRODUCTION

Although pain is highly prevalent among the elderly and they are amongst the highest users of analgesics, clinical evidence to support evidence based treatment decisions is limited (1). Pharmacokinetic (PK) and pharmacodynamic (PD) data on analgesics in elderly patients, especially those older than 75 years, is sparse, yet this data is critical to ensure safe use of these medications in this population (2-7). Furthermore, less than 10% of drug delivery technology trials conducted included PK assessments in the elderly. As recently as 2014, a systematic review of clinical trials in low back pain found that elderly adults older than 65 were systematically excluded from randomised clinical trials and that, despite calls to include elderly subjects in such studies, there has been no increasing trend between 1992 and 2010 (7).

Tramadol hydrochloride, a weak centrally acting analgesic structurally related to morphine and codeine, is widely recommended to treat moderate to severe pain in a variety of chronic conditions that affect elderly patients including osteoarthritis (8), low back pain (9) and neuropathic pain (10). Tramadol has a unique mechanism of action with both opioid and non-opioid related analgesia (5). Commonly used pain relievers such as ibuprofen and naproxen are non-selective cyclo-oxygenase inhibitors and carry a significant risk of cardiovascular events including death, gastrointestinal bleeding and kidney dysfunction and are therefore used with extreme caution or not at all in the elderly. These side effects are generally not associated with use of tramadol and other opioids, making them an option for older patients with chronic pain (11). Tramadol most frequently documented adverse effects in clinical and post-marketing surveillance studies were nausea/vomiting, dizziness, drowsiness, tiredness, sweating and dry mouth (12). However, older adults are at increased risk of seizures with tramadol use, nearly 25% of new seizures occurring in patients aged 65 or older (13). The potential for seizures and serotonin syndrome even within tramadol recommended dosing range and the potential for clinical utility in the elderly highlight the importance of understanding any difference in the PK and/or PD of tramadol and its active metabolite, (+/-)-O-desmethyltramadol (ODM).

Racemic tramadol, its enantiomers and the ODM metabolite, are implicated in the production of antinociception through both opioid and non-opioid mechanisms (14). Tramadol acts as an opioid agonist by selectively and weakly binding to μ-receptors in the spinal cord and brain, (+)-tramadol with greater affinity than (-)-tramadol (15, 16). The (+)-ODM metabolite, however, has 200 times the affinity of the racemic molecule. As a result, the opioid action of tramadol is thought to be primarily linked to the (+)-ODM metabolite (17, 18). Tramadol is also suggested to have enantioselective analgesic activity through descending inhibitory pathways by means of (+)-tramadol inhibition of serotonin reuptake (5-HT) and (-)-tramadol inhibition of norepinephrine (NE) reuptake; however, ODM appears to be inactive (19, 20).

Following oral administration, tramadol mean apparent total clearance was 45 l/h and mean elimination half-life 5 hours; absolute bioavailability was estimated as 68%, mostly due to hepatic first-pass effect (21). Tramadol is approximately 20% plasma protein bound. Renal elimination accounts for 90%, the remainder of a radioactively labelled dose being recovered in faeces (22). Tramadol is metabolised in the liver. CYP2D6 enzymes are responsible for the formation of ODM (22, 23). Since the (+)-ODM metabolite is the main activator of the opioid mechanism of action of tramadol, CYP2D6 plays an important role in analgesic response with tramadol. CYP polymorphisms may be the source of variability in individual PK and PD parameters with tramadol (23, 24).

In the elderly, tramadol pharmacokinetics has been poorly characterized. In a review citing data on file with the manufacturer, Lee et al. reported that, since age-related pharmacokinetic changes did not reach statistical significance, dose reduction in elderly patients with relatively normal renal and hepatic functions was not considered necessary (6). Likar et al. found that steady-state plasma concentrations of tramadol and (+)-ODM, collected at 2 time points during the dosing interval, showed no age-related differences (25).

It has been suggested that research on analgesic efficacy in humans can be enhanced by using human experimental pain models which offer the opportunity to assess human responses to

pain in a more controlled setting using objective measures. Tramadol PD has been examined in a variety of experimental pain models including those that utilise transcutaneous electrical stimulation, dry air and $CO_2$ to the nostril and electrical stimulation of the tooth pulp (26-29). With the exception of the last, results demonstrated an analgesic effect. The studies utilised both immediate release (IR) and slow release (SR) formulations which may have cause differences in onset and duration of analgesia as well as time to peak effect. Nonetheless, in general onset of analgesia occurred within 2 hours and duration of analgesia was 6-12 h depending on the dosing interval of the formulation. In a study by Sarbu et al. (30), 47 patients with acute low back pain were administered a single 200 mg extended release tramadol tablet (intended for once daily administration). Onset of perceptible pain relief was achieved within 1 hour for the majority of patients and at plasma levels suggesting a therapeutic threshold between 50 and 100 ng/ml.

Studies of the PD of tramadol in the elderly are rare. Likar et al. (25) conducted a fixed sequence active comparator study examining the impact of treatment with tramadol IR and SR on 100 patients with previously existing painful conditions. Patients were stratified by age group (< 65 years, 65-74 years and 75 years and older) to ensure similar baseline pain intensity amongst the groups. Pain was reported on three scales: the 100 mm VAS, the 11-point PNRS and a 4-point verbal rating scale. They found no age-related differences in any of the pain rating scales.

Electrically Stimulated Pain Models (ESPMs) can be used to selectively activate different afferents and nervous structures and thereby evoke various pain sensations (31). In a previous noncompartmental analysis of the PD data presented herein, EPSM was able to detect a difference in pain tolerance at 5Hz frequency between tramadol and placebo treatment in elderly subjects (32). However, it was impossible to delineate whether this increase in pain tolerance was due to a decrease in pain sensitivity or to an increased exposure to (+)-ODM in the elderly.

***Study objectives***

The analyses presented here are intended to provide exploratory data to describe any age-related differences in the concentration-effect relationship of (+)-ODM, the active metabolite of tramadol, using the PTT as a biomarker for analgesic effect.

## 2 MATERIALS AND METHODS

### 2.1 Experimental design

This was a two-cohort, randomised, double-blind, placebo-controlled, two-period crossover, PK/PD study using an electrically stimulated pain model (ESPM). Subjects received either a single oral dose of 200 mg Tramadol Contramid® OAD controlled-release tablets or matching placebo with a 7-day washout between each period. The study was conducted between January and February 2007 at a phase I facility where subjects were confined for 12 h prior to dosing and for 48 h afterwards. One of the objectives was to evaluate the PK/PD relationship of (+)-ODM after a single dose of tramadol in elderly (≥ 75 years) and healthy young (18-40 years) volunteers. Before initiation of any study-related procedure, the protocol and informed consent were reviewed and approved by two independent ethics committees (Comité d'Éthique de la Recherche des Sciences de la Santé, Université de Montréal; and Investigational Review Board, MDS Pharma Services, Montreal) and written consent obtained from subjects. The study was conducted in accordance with the Declaration of Helsinki as well as the Enoncé de politique des trois Conseils.

### 2.2 Subjects

At screening, subjects were determined to be healthy based on medical history, physical examination, and evaluation of vital signs, electrocardiogram (ECG), and clinical laboratory data. Subjects were genotyped and those with the poor metabolizer variant of the CYP2D6 gene were excluded to minimize intra-group variability and inter-group differences not related to age. Potential subjects with a body mass index (BMI) > 35 kg/m$^2$ or those who had donated blood frequently in the previous year were excluded, as were subjects with an increased risk of seizures, with bowel disease affecting absorption or previous failure of treatment with

tramadol or discontinuation of treatment due to adverse events. Female subjects of childbearing potential had to have negative pregnancy test results at screening and clinic check-in for each study period. Subjects abstained from taking substances known to be strong inhibitors of CYP isoenzymes within 10 days, or inducers of CYP isoenzymes within 28 days, prior to dosing. Use of all medication (including over-the-counter products) was prohibited for 7 days prior to dosing and during the time of sample collection with two exceptions: elderly subjects were permitted to continue taking stable doses of chronic medications, other than strong CYP inhibitors/inducers, and female subjects were permitted to continue taking hormonal contraception or replacement therapy. Use of concomitant medications was recorded.

## 2.3 Data

### 2.3.1 Database

PK and PD data from this analysis were from a single phase I study (Clinicaltrials.gov identifier:NCT02329561). All plasma concentrations underwent a quality control check prior to database lock. A dose of 87.84 mg of the (+)-enantiomer of tramadol (base form) was used as the dose input. The model was based on (+)-ODM concentrations since it has been found that, when modeling downstream metabolites, fitting the active moiety concentrations alone results in a simpler, more efficient model and yields similar predictions to a model that includes parent concentrations. All recorded data from the PD evaluations were entered into Microsoft Excel 2010® (Microsoft Corporation, Redmond, WA) and double verified for accuracy. Initial cleaning of the database to remove duplicates and obvious outliers (33) and initial establishment of baseline was conducted prior to un-blinding of the data.

## 2.4 PK evaluations

### 2.4.1 Sample collection

Blood samples were collected prior to the time of dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 20, 24, 30, 36, 42 and 48 h post-dose. Blood collection was carried out by direct venipuncture

or butterfly catheter into lithium heparin collection tubes. Samples were immediately cooled in an ice-water bath and centrifuged under refrigeration. Plasma samples were then divided into two aliquots and stored at -20 ± 10°C, pending assay.

*2.4.2 Bioanalytical method*

Plasma concentrations of tramadol and ODM-metabolite enantiomers were measured by high performance liquid chromatography (Agilent 1100) using a Chiralpak® IA (250x4.6 mm, 5µm) analytical column maintained at 5°C (34). The mobile phase consisted of acetonitrile:water:diethylamine (950:50:0.1; v/v/v) delivered at a rate of 0.6 ml/min. Elution times were within 9 min for all analytes. A triple quadrupole mass spectrometer (Applied Biosystems, API 4000) at unit resolution in the multiple-reaction-monitoring mode was used to monitor the transition of the protonated precursor ions to the product ions by the Turbo V electrospray interface (ESI). Transitions were *m/z* 264-58 for tramadol enantiomers, and *m/z* 250- 58 for ODM enantiomers. Main parameters were the following: source temperature 650°C, ion spray voltage 5.25 kV, declustering potential 46 V. MS/MS parameters were collision energy 51 eV and collision gas pressure ($N_2$) 6 mPa.

Plasma (0.3 ml) samples were vortexed after successive addition of internal standard (ketamine) and 1M sodium carbonate buffer (0.1 ml, pH 9); then extracted with 3 ml of hexane:chloroform (3:2) by vortexing for 5 minutes before centrifugation. The aqueous phase was flash frozen in an alcohol bath (-18°C) and the organic phase decanted, evaporated to dryness under nitrogen at 40°C and reconstituted with 5 ml of acetonitrile:water:diethylamine (50:50:0.1; v/v/v) by vortexing for 30 seconds. Injection volume was 5 µl.

The method was validated by FARMOVS-PAREXEL Clinical Research Organisation, (Bloemfontein, South Africa) according to procedures and acceptance criteria recommended for bioanalytical method validation for PK studies (35). Matrix effects were lower than the linear range. Plasma calibration curves fitted a Wagner regression over the ranges of 3.126-400.1 ng/ml for (+)- and (-)-tramadol and 1.563-200.0 ng/ml for (+)- and (-)-ODM. Mean efficiencies of extraction were 81% (CV 3.1%) and 66% (CV 3.2%) for tramadol and ODM

enantiomers, respectively. Mean inter-day accuracy ranged between 98.2 and 102.0 % with a maximum CV for precision of 6.9% for all analytes.

### 2.5 PD evaluations

As previously described (32) data on Pain Tolerance Threshold (PTT) were collected using a 5 Hz stimulus applied to the non-dominant middle finger with two 1-cm diameter gold-plated surface electrodes linked to the Neurometer® CPT/C (Neurotron, Inc., Baltimore, MD, USA). The Neurometer® is a fully automated quantitative neurodiagnostic device that generates constant alternating current sinusoid waveform stimuli at 5 Hz and has a possible range from 1 to 1000 μA (automatic cut-off) (36-38). The Neurometer® was used to measure PTT which was defined as the maximum amount of atraumatic neuroselective electrical stimulus that a volunteer was willing to tolerate. Electrical stimulation procedures were conducted at the following times prior to dosing and at 0.33, 0.75, 1.25, 1.75, 2.5, 3.5, 4.5, 5.5, 7, 9, 11, 14, 20, 24 and 30 hours after dosing.

#### 2.5.1 PK/PD analysis

Simultaneous population PK/PD modeling of (+)-ODM was conducted using Phoenix® NLME version 7.0 (Certara USA, Inc., Princeton, NJ). Based on previous population PK modeling for tramadol (34), a one compartment model for the PK of (+)-ODM was chosen. The base model was developed using first order conditional estimation with interaction (FOCE-ELS). An exponential model was used to characterise between subject variability (BSV) for both the PK and PD parameters (Eq. (1)). This model was assumed to be normally distributed around the typical value for the population:

$$P_{ij} = \theta j \cdot exp(\eta_{ij}) \qquad (1)$$

Where $P_{ij}$ is the $j^{th}$ parameter value for the individual $i$, $\theta j$ is the $j^{th}$ typical parameter value for the population and $\eta_{ij} \sim N(0, \omega_j^2)$ where $\eta_{ij}$ is a random variable for the $i^{th}$ individual and

the $j^{th}$ pharmacokinetic parameter distributed with a mean of zero and a variance of $\omega_j^2$ for that parameter. An additive model was used to characterise the residual error due to the need to permit negative values in the PD parameters. $E_{max}$ and sigmoid $E_{max}$ models were tested.

Final model selection was based on the inspection of residuals, -2log-likelihood (-2LL) and visual predictive checks. Visual predictive checks were used to evaluate the performance of the model by comparing the $5^{th}$ and $95^{th}$ percentile of the simulated effect versus time curves to the observed data. The full model was tested using a parametric bootstrap with 1000 random samples to determine the bootstrap median and 95% CI for each of the parameter point estimates.

Likely covariates such as age, age group and period were tested in the full model. The influence of the covariate was considered significant if the difference between the means of parameter with and without the covariate fell within the 2.5 to 97.5 percentile bootstrap confidence interval and did not include zero (33, 39, 40).

*Final model*

A single compartment PK model parameterised with a first-order formation rate constant ($k_m$), apparent volume of distribution of the metabolite ($V/f_m$) and clearance of the metabolite ($Cl/f_m$) was used to describe (+)-ODM concentrations (Eq. (2)) and was fit simultaneously with the PD data.

$$A_m(t) = (-Cl/f_m \cdot A_m) \,.\, Dose /k_m \tag{2}$$

$$C_m(t) = A_m(t) /V/f_m \tag{3}$$

Where $A_m$ is the amount in the central compartment, $C_m$ is the plasma concentration of the (+)-ODM metabolite and Dose is the (+)-tramadol portion of the tramadol dose given.

A sigmoid $E_{max}$ PD model which accounted for baseline and placebo periods was determined to best describe the relationship between the PK and PD data (Eq.(3)).

$$E = E_0 + E_p + \frac{E_{max} \cdot C_m^{\gamma}}{C_{m50}^{\gamma} + C_m^{\gamma}} \quad (3)$$

Where $E_0$ is the baseline PTT value and $E_p$ represents the placebo effect. The drug effect was modeled as an sigmoid $E_{max}$ function where $E_{max}$ is the maximum PTT, $C_{m50}$ is the plasma concentration of (+)-ODM corresponding to 50% of the maximum PTT, $C_m$ corresponds to the plasma concentration of (+)-ODM and $\gamma$ is the shape parameter which was fixed to an estimated value to improve the stability of the model.

Placebo was modeled as a linear time-independent function (Eq. (4)).

$$E_p = \text{Intercept}_p \quad (4)$$

Finally, covariate testing determined that age should be added as a covariate on $Vd/f_m$, $Cl/f_m$ and $E_{max}$ while period was added as a covariate only on baseline PTT value.

Diagnostic plots and visual predicted check (VPC) were carried out for model validation and bootstrap analysis for model robustness.

## 3 RESULTS

Subject baseline characteristics and demographics are summarised in Table 1. A total of 20 young and 15 elderly subjects were enrolled in the study. One subject from the elderly group discontinued early in the first period due to personal reasons and was excluded from the analyses. Five subjects, 4 from the young group and 1 from the elderly group, were excluded from analyses due to a food effect (34). Thus, analyses presented here include 29 healthy young and elderly subjects most of whom were male; baseline characteristics and demographics are presented in Table 1. Adverse events experienced by at least 10% of patients are presented in Table 2 for young subjects (n = 20) and elderly subjects (n=15) by

active versus placebo administration. The adverse events experienced at least 10% of young and elderly subjects under active administration were nausea, dizziness, vomiting and somnolence. No adverse events were experienced in young subjects under placebo administration while 1 patient experienced dizziness and nausea in the elderly group when administered placebo. Mean vital signs data (respiratory rate, pulse, blood pressure and temperature) were within normal limits, with minor changes from baseline. Occasionally individual post-dose blood pressures or pulses were transitorily out of range but quickly returned to normal in both age groups. With the exception of one elderly male subject (80 years old) whose systolic blood pressure was high at baseline and throughout the study. Some elderly subjects had Electrocardiogram (ECG) results at baseline and throughout the study but these abnormalities were not considered clinically significant. In the first cohort of patients, a concealed electrical panel at the research clinic interfered with the functioning of one of the neurostimulation devices by spontaneously shutting it down at times before PTT was reached. The issue was resolved by the time the second cohort was brought to the clinic for testing. Despite this, there was no statistically significant cohort effect.

Mean plasma concentrations of (+)-ODM calculated using a noncompartmental PK analysis were presented in an earlier publication (34) That analysis showed that (+)-ODM peak levels occurred later and concentrations declined more slowly in the elderly than in young subjects.

Population pharmacokinetic and pharmacodynamics parameters are presented in Table 3. The 95% CI for the difference between young and elderly did not include 0 for both $Vd/f_m$ and $Cl/f_m$, suggesting that there may be a true difference between the elderly and young, $Vd/f_m$ being 76% larger in the elderly and $Cl/f_m$ being 16% slower . At baseline ($E_0$) pain tolerance was similar between young and elderly subjects. However, the $E_{max}$ parameter was 2.5 times higher in the elderly. Indeed, we computed the maximum possible treatment-related effect by summing $E_0$, $E_{pbo}$ and the $E_{max}$ parameter with an effect for age group. The maximum possible effect was 169 [135 - 221] in the young and 194 [149 - 252] in the elderly; 15% higher in the elderly. There was no difference between the age groups with regard to $E_0$ and $C_{50}$ (Table 3).

Diagnostic plots for the final model show that the predictions for (+)-ODM and PTT matched the observed data satisfactorily (Figure 1) and furthermore, that residual errors were approximately normally distributed over time and predicted concentration and effect adequately fitted data. The VPC show that the final model adequately captured the central tendency and spread of the both the concentration and effect data for which the data for both mostly lie within the 95% CI (Figure 2)

## DISCUSSION

Tramadol is an analgesic that is widely used to treat pain in conditions that predominantly affect elderly patients yet our knowledge of its PK and PD in the elderly has been limited. An understanding of the PK/PD of (+)-ODM, the active metabolite, in the elderly is important to safe prescribing and use to ensure that age-related differences are taken into account. The results of this exploratory analysis suggest that even in relatively healthy elderly subjects there are differences. Distribution and elimination processes appear to be different for (+)-ODM, with $Vd/f_m$ being 76% larger and $Cl/f_m$ being slightly slower (16%), resulting in the potential for greater exposure to the metabolite in the elderly. Baseline ($E_0$) and sensitivity ($C_{m50}$) for pain tolerance were similar between young and elderly subjects. Although, maximum tolerance to painful stimuli ($E_{max}$) is increased by 60% in elderly the increase in overall treatment-related maximum effect is less (15%).

The results of this population PK/PD analysis are in general agreement with findings from our previous non compartmental analysis of (+)-ODM PKs in the elderly (34) where we found that (+)-ODM $AUC_{0\text{-}inf}$ was approximately 30% higher in elderly subjects and where mean renal clearance was statistically lower in the elderly by 26%. However, the present analysis indicates a 76% increase in the ODM apparent Vd/fm. In agreement with results found from our previous PopPK analysis of tramadol (34), the only significant covariate found was age on volume of distribution. This significant increase cannot be explained by a decrease in ODM formation, according to the amount recovered in urine (34). Similar correlations were found for ODM herein and these changes in distribution would most likely be responsible for the

60% increase in maximum PD effect observed in the elderly ($E_{max}$). In contrast to tramadol, there is a significant age-related decrease on Cl/fm (16%) but lower than that reported in our previous NCA (30%).

The present PK/PD analysis appears to show that a 15% higher maximum possible treatment-related effect may be associated with the higher systemic exposure to ODM. This can be explained by the highly variable PT values observed during the placebo period and required the use of a time independent function.The treatment-related effect during the placebo period was quite close to the baseline value ($E_0$). This is in agreement with was observed in our previous noncompartmental analysis of PD data where LSM of $E_{max}$ during the active treatment period was increased by 25% in the elderly compared to the young (Submitted to Pain Physician).

Sensitivity of PTT to (+)-ODM ($C_{m50}$) was not statistically different in the elderly. This finding is in agreement with other PK/PD studies carried out for other CNS drugs in the elderly. Literature suggests that age-related differences in sensitivity to pain may be related to changes in A-δ fibres. After thermal noxious stimuli, myelinated A-δ fibres show reduced pain perception and longer sensory evoked potentials while both parameters remaine unchanged for unmyelinated C-fibres. This apparent discrepancy is possibly due to reduced density and function of myelinated fibres, including structural modification and reduced conduction velocity with age (41, 42). Tseng et al. (43) found a reduction in the sensory areas of the brain activated and the magnitude of the activation in the elderly using functional magnetic resonance imaging after noxious thermal stimulation. This may be why we did not see any difference between young and elderly in sensitivity.

Studies of the analgesic effect of tramadol after use of a percutaneous electrical stimulation and cold pressor experimental pain models in CYP2D6 poor and extensive metabolisers have demonstrated greater analgesic effect among extensive metabolisers than among poor metabolisers, although poor metabolisers still achieved analgesia, possibly as a result of the non-opioid mechanisms of action of the parent compound (43, 44). Binding to the μ-opioid

receptor is associated with a variety of biological effects including the desired effect of analgesia. It is possible that the higher tolerance to EPSM seen in elderly subjects is a result of the greater exposure to (+)-ODM. Whether or not this would translate into greater efficacy in patients is speculative. It also raises the potential that there could be a higher occurrence of a variety of undesirable biological effects. This was not the case in our study where young subjects had a higher number and percentage of adverse events; however our study involved administration of a moderate single dose of 200 mg of tramadol (approved dose range 200-400 mg) to relatively healthy elderly subjects and the number of subjects enrolled is too low to draw conclusions about safety of tramadol. This is especially true in the context of the many years of safety data collected since tramadol was first approved for use. Although, there is little information on occurrence and rates of events in elderly patients compared to young patients. Likar et al. (25) reported adverse events from 30 subjects 75 years and older who received multiple doses of tramadol for moderate to severe pain. Adverse events led to discontinuation in 4 patients (12%). The most common events reported in the Likar study are the same as those in our study. Adverse events experienced in 10% or more of these patients included nausea (10 (26%)), dizziness and giddiness (10 (26%)), vomiting (6 (19%)) and constipation (5 (16%)) and malaise and fatigue (3 (10%)). Several authors have suggested that tramadol should be used with great care or not at all in elderly subjects due to a high incidence of μ-opioid receptor related side effects (44) or frequency of drug-drug or drug-disease interactions and variability in efficacy and side effects (1). In the U.S. in 2011, 35% of emergency room (ER) visits involving adverse reactions to tramadol were undertaken by older adults (aged 65 and older) (13). Of all tramadol related adverse events that resulted in an ER visit 17% resulted in hospitalisation and half of those were in adults 65 years or older (45).

The findings of our noncompartmental analysis suggest that there is a 30% increase in exposure to (+)-ODM in the elderly subjects as compared with young (34). This raises the concern that accumulation could occur, although based on this data the accumulation factor is low (1.1), suggesting that dose adjustment in relatively healthy elderly patients with mild renal impairment is likely unnecessary. In young subjects, after hepatic biotransformation, tramadol and its metabolites are largely eliminated by the kidneys (~90%) with the remainder

eliminated in feces. Our previous study showed that 20% of a tramadol dose is excreted unchanged and 15% as ODM (34). In its guidance on pharmacokinetics in patients with impaired renal function, the U.S. Food and Drug Administration recommends that dose adjustment should occur in patients with renal insufficiency in medications that are excreted more than 50% unchanged (46). This is not the case with tramadol, so it is unlikely that purely on that basis, dose adjustment would be recommended. However, the trend we observed suggests that an awareness of the renal status of elderly patients taking tramadol is important and that careful consideration should be given to using tramadol in patients with the potential for greater levels of renal impairment, the potential for hepatic impairment and with frailty. The classic instruction to start with low doses of tramadol and titrate carefully to balance benefit and risks of the treatment would appear to be a wise approach.

Clinically the differences we saw in elderly subjets could result in slower onset of analgesia, greater efficacy but also greater risks of side effects and a greater potential for accumulation. That being said the differences in the relatively healthy population of elderly volunteers from this study as compared to the healthy young subjects in this study are unlikely to result in clinically significant consequences. Nonetheless, in elderly patients with comorbid diseases, multiple medications and greater hepatic and renal impairment there could be clinically significant increased exposure to (+)-ODM and resultant increased risk of side effects; both of which could require using lower doses in these patients.

The results obtained from an experimental pain model conducted in volunteers may have limited generalizability to treatment of elderly patients. However, Olsen et al. suggest that experimental pain models offer the opportunity to study pain responses when they are not blurred by other symptoms and where confounding environmental circumstances are controlled (31). Ideally, this study would have included a greater number of elderly subjects and a better balance amongst the sexes. Age was not treated as a covariate but as a binomial.

The inclusion of an older group of young subjects, for example 25 to 40 years, may have allowed this variable to be continuous and more powerful.

Future work should incorporate subjects with a greater degree of renal insufficiency, since study entry was limited to subjects with mild renal insufficiency, it does not reflect the situation of frail elderly persons. Although the study permitted subjects with mild hepatic insufficiency, none of the elderly volunteers had hepatic insufficiency, which could also have given information about frailer elderly subjects.

## CONCLUSIONS

This exploratory analysis suggest that age-related differences in distribution and elimination processes exist for (+)-ODM resulting higher exposure associated with an increase in maximum tolerance to painful stimuli ($E_{max}$) of 60% in elderly with a lower difference in overall treatment-related maximum effect (15%). This carries the potential for greater efficacy at the same dose in elderly subjects but also more side effects.

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**Table 1. Baseline characteristics and demographics**

| | Young (18 - 40 years) n = 16 | Elderly ( ≥ 75 years) n = 13 |
|---|---|---|
| Age (years) | | |
| Mean ± SD | 28 ± 6 | 77 ± 2 |
| Range | 21 - 38 | 75 – 80 |
| Sex n (%)[a] | | |
| Male | 13 (81) | 10 (77) |
| Female | 3 (19) | 3 (23) |
| Weight (kg) | | |
| Mean ± SD | 74 ± 10 | 78 ± 7 |
| Range | 59 – 98 | 65 – 93 |
| BMI ($kg/m^2$)[b] | | |
| Mean ± SD | 25 ± 2.03 | 28 ± 2.66 |
| Range | 21 – 27 | 25 – 35 |
| GFR ($mL/min/1.73m^2$)[c] | | |
| Mean ± SD | 103 ± 14 | 68 ± 12 |
| Range | 78 – 135 | 50 – 90 |

SD: Standard Deviation; BMI: Body Mass Index; GFR: Glomerular Filtration Rate

[a] Percentage of subjects who are male or female within the age group

[b] The difference in BMI between the age-groups was statistically significant ($p<0.001$)

[c] GFR was calculated using serum creatinine according to the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula. The difference between the age groups was statistically significant ($p<0.001$)

**Table 2 Most commonly reported adver00se events[a] by age group and active or placebo treatment all subjects exposed[b] (34)**

| Adverse event [b] | Young n=20 | | Elderly n=15 | |
|---|---|---|---|---|
| | Active | Placebo | Active | Placebo |
| Nausea | 9 (45) | 0 (0.0) | 2 (10) | 1 (7.1) |
| Dizziness | 7 (35) | 0 (0.0) | 3 (15) | 1 (7.1) |
| Vomiting | 5 (25) | 0 (0.0) | 3 (15) | 0 (0.0) |
| Somnolence | 2 (10) | 0 (0.0) | 2 (10) | 0 (0.0) |

[a] Adverse events reported by 10% or more of patients

[b] Adverse events presented here are from all subjects exposed in the study, whereas the analysis presented here exclude 1 subject who discontinued early for personal reasons and 5 who were excluded from the analysis due to a food effect (see Results)

[c]Number and percentage of subjects experiencing the adverse event at least once

**Table 3. PK/PD Model of (+)-ODM and 5 HZ PTT in young versus elderly subjects: bootstrap model parameters**

| Parameter | Young<br>n = 16<br>*Typical Value*<br>Bootstrap<br>Median<br>[95% CI] | Elderly<br>n = 13<br>*Typical Value*<br>Bootstrap<br>Median<br>[95% CI] |
|---|---|---|
| $K_m$ ($h^{-1}$) | *0.099*<br>0.100<br>[0.082 - 0.121] | *0.175*<br>0.166<br>[0.093 - 0.265] |
| $V/f_m$ (l) | *979*<br>981<br>[889 - 1082] | *1728*<br>1648**<br>[1170 - 2244] |
| $CL/f_m$ (l/h) | *116*<br>116<br>[107 - 128] | *97*<br>96**<br>[85 - 113] |
| $E_0$ (μA)* | *143*<br>143<br>[113 - 180] | *132*<br>132<br>[93 - 166] |
| Emax (μA)* | *24*<br>*25*<br>[10 - 45] | *60*<br>61**<br>[36 - 95] |
| $C_{m50}$ (ng/ml) | *19*<br>18<br>[9.00 - 21] | *14*<br>13<br>[6.00 - 15] |
| $E_{pbo}$ (μA)* | 0.95<br>1.00<br>[0.77 - 3.06] | 0.95<br>1.00<br>[0.77 - 3.06] |

Note: Bootstrap – Median and 95% CI estimated by applying final PK/PD model to 1,000 resampled data sets; Km- constant of formation of the metabolite(m), V/fm – apparent volume of distribution; Cl/fm – apparent clearance; $E_0$ - estimated overall baseline; $C_{m50}$ – sensitivity of PTT to (+)-ODM; $E_{pbo}$ - estimated placebo effect

* Maximum possible treatment-related effect was computed as the sun of $E_0$ +$E_{pbo}$+ $E_{max}$ and by taking into account the effect of age: young: 169 [135 - 221] and elderly: 194 [149 - 252].

** 95% CI for the mean difference young versus elderly did not include 0 suggesting that there may be a difference

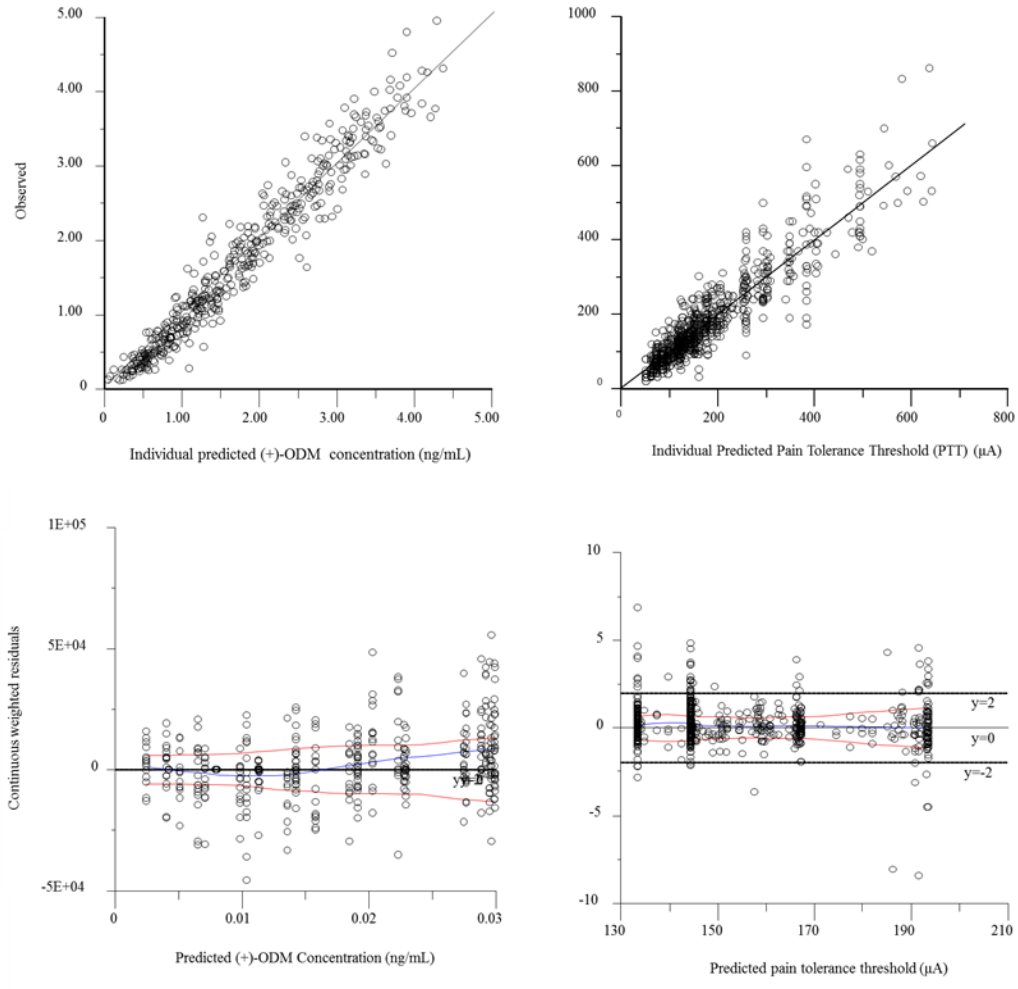


**Figure 1. Diagnostic plots for goodness of fit of the final PK/PD model**

Circles represent individual concentration and PTT values, Upper panels are the observed concentration (left graph) and pain tolerance (right graph) versus individual model predictions; the solid line represents the identity line. Lower panel is the continuous weighted residuals versus predicted concentrations (left graph) and pain tolerance threshold (right graph).

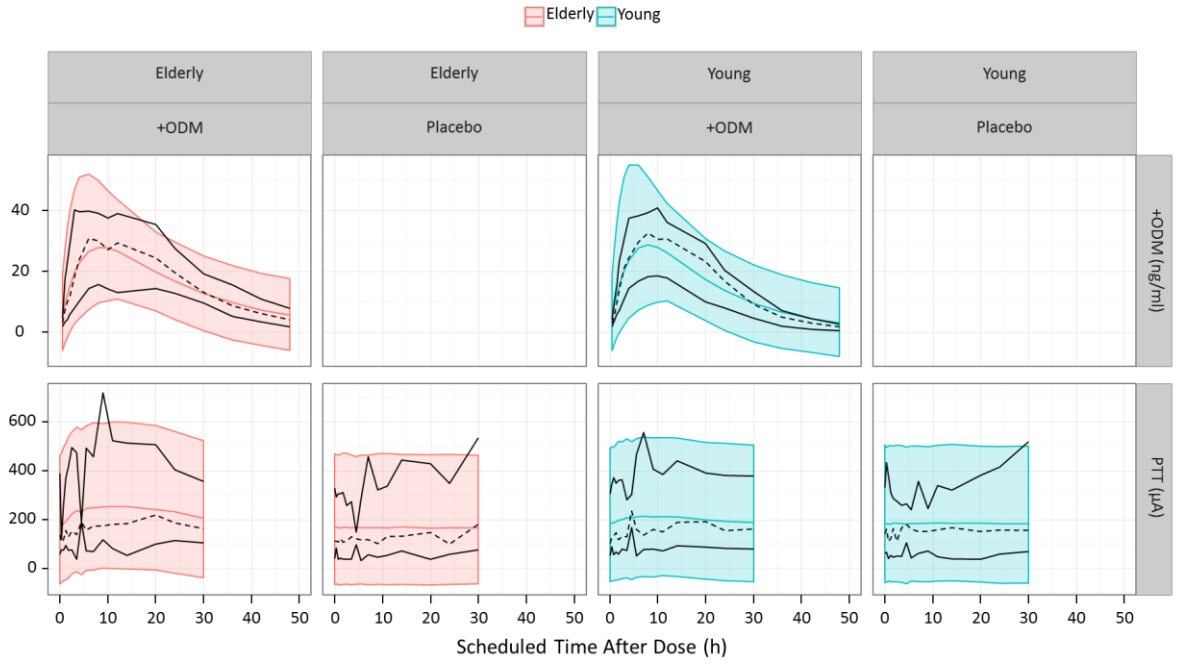


**Figure 2. Visual predictive checks of the time course of (+)-ODM concentration and PTT in young and elderly subjects**

Note: The upper panel represents (+)-ODM plasma concentration versus time while the lower panel represents the pain tolerance on the left under active treatment and on the right under placebo treatment.

The black lines represent the model estimates and 95% CI with dotted line represeting the mean typical value. The shaded areas represent the 95% CI of the model simulations (N=1000), the middle solid line represents the median estimate from the bootstrap.

# 7. General Discussion and Conclusions

## 7.1 General Discussion

Thanks to improvements in medical care, populations around the world are aging as people survive many of the illnesses or accidents that in the past might have shortened their lives; this carries with it the challenge of ensuring that in addition to surviving, people live quality lives as they age and that health care systems are able to sustain care for people into their old age (1, 4, 7, 34, 162). Developed economies are already struggling to meet this need and it will be an even greater challenge in countries and regions where health care resources are limited. Regardless all health care systems will be challenged to use their resources wisely. Understanding the PK and PD of medicines used to treat conditions that commonly affect elderly people is key to treating them effectively, allowing them to live with quality of life and dignity and minimising the side effects that can interfere with these first two goals. Pain is a condition that increases with age yet there is little information about the PK and PD of many analgesics in elderly patients (1-3, 7, 10, 162).

Tramadol is one of the analgesics commonly used to treat osteoarthritis, back pain and neuropathic pain, all of which are present in elderly patients (92, 97, 163-166). Although there has been some concern expressed about its use in elderly, there is little information about age-related differences in its PKs and PD. Tramadol was first approved for use in Germany in 1977. At that time, regulatory requirements for approval required very limited data in elderly subjects. Since then the EMA has issued guidance on development of medications in elderly and special populations, but the evidence requirements continue to lag behind those for pediatric use of medications. Thus, treatment decisions in elderly patients continue to be made based on less than ideal evidence. We should be generating evidence to tailor pharmacotherapy to the needs of the elderly, taking into consideration the decline of different physiologic processes, to improve both efficacy and reduce the risk for adverse drug events through prediction (167). Indeed much can be learned and applied from the approach taken in

pediatric medicine development, particularly regarding the use of pharmacometrics in gathering evidence in populations that are small or difficult to recruit into clinical trials.

The objective of this thesis is to contribute to the knowledge about age related differences in the PK and PD of tramadol and its active metabolite ODM in subjects 75 years and older in order to determine whether there are age related differences. We did this by conducting in depth PK analysis of (+)- and (-)-tramadol and (+)- and (-)-ODM plasma and urine concentrations as well as a population PK analysis of tramadol. Subsequently, in anticipation of a PK/PD population analysis, we utilised data resulting from transcutaneous electrical stimulation of the middle finger with painful stimuli at 250 and 5 Hz to validate the experimental pain model (EPSM), select the most sensitive stimulus for PTT and explore any age related differences in PTT. Finally, utilising plasma concentrations of (+)-ODM and the PTT data from the 5 Hz stimulus, we conducted a population PK/PD analysis to determine any age related effects on the PK and PD of (+)-ODM. To our knowledge this is the first population PK/PD analysis of (+)-ODM in patients 75 years and older.

### *Characterisation of the PK of both enantiomers of tramadol and O-desmethyl tramadol in healthy young (18-40) and elderly subjects (75 years and older)*

#### Age related differences

We utilised two analysis approaches to characterise the PK of tramadol and O-desmethyltramadol and their enantiomers in young and elderly patients after a single dose of once daily tramadol 200 mg. The NCA demonstrated comparable $C_{max}$ and AUC between age-groups for tramadol enantiomers; however, there were significant differences in $V_{area}/F$ (mean 34% higher) and $k_{el}$ (mean 28% lower) in the elderly. In addition the PK of ODM was significantly different in the elderly for $AUC_{0\text{-}inf}$ (mean 35% higher), $Clr_{0\text{-}48}$ (mean 29% lower) and $k_{el}$ (mean 33% lower). In the population analysis, which examined tramadol PK, we examined all likely covariates such as age, sex, glomerular filtration rate (GFR), and food-effect (FE) that might result in differences in disposition, metabolism and elimination in elderly subjects as compared to young subjects. In the end, the population analysis identified age as a covariate only of V/F (Young: 305 L; Elderly: 426 L) with a 50% longer mean elimination half-life in the elderly. The mean total amount recovered in urine was not

statistically different between age groups for tramadol or ODM nor was the metabolic clearance to ODM different. No differences in absorption processes were observed. Thus tramadol exposure was similar between the age-groups; but exposure to ODM was higher in elderly subjects. An important limitation of this data was that we did not have PK data after I.V. administration of tramadol; therefore we had to assume that there were no differences between young and elderly subjects in bioavailability of tramadol and ODM. The age related differences in the PK of the enantiomers of ODM were statistically significant and are potentially clinically significant, warranting further examination, particularly since (+)-ODM is considered responsible for the opioid efficacy and opioid-related side effects of tramadol.

**Stereoselective PK**

Tramadol is typically a racemic mixture of (+)- and (-)-tramadol (72), therefore a 1:1 ratio between (+)- to (-)- tramadol enantiomers in the tablets was assumed during PK analysis but no quantitative determination was performed. The population analysis of tramadol was conducted by summing the plasma concentration for the (+)- and (-)- tramadol enantiomers. The NCA was conducted on the individual enantiomers of both tramadol and ODM. Both tramadol and ODM demonstrated stereoselective pharmacokinetics. For within age-group comparisons between enantiomers exposure was approximately 20% higher to (+)-tramadol while half life and renal clearance were similar, while for (+)-ODM renal clearance was approximately 30% higher but exposure was also similar.. This is consistent with both *in vitro* and *in vivo* findings in the literature (99). The difference between the age-groups in the enantiomeric ratios for both T and ODM were not statistically significant, suggesting that generally there is no age related stereoselectivity.

These PK analyses make an important and unique contribution to our knowledge about tramadol and ODM in the elderly. Our results, showed no difference in the mean total amounts of tramadol and ODM recovered in urine. Furthermore, there was no difference in the metabolic clearance to ODM. These two findings suggest that age related decline in hepatic clearance of tramadol by means of CYP2D6 mediated O-demethylation is less

pronounced than the age related decline in renal clearance of tramadol and its metabolites. Allegaert (167) noted the similarities in our findings and those in studies of tramadol early infancy where the maturational increase in hepatic drug metabolism (clearance to ODM) capacity is faster compared to the maturation of renal elimination (renal ODM elimination) capacity (168). In both populations, the difference in age related changes in hepatic and renal clearance results is proportional to the increase in ODM exposure, and may explain increased sensitivity to (side) effects.

Age-related changes in hepatic function seem to be primarily related to reduction in hepatic blood flow, the evidence for differences in Phase I metabolism is inconsistent and there is to date no evidence for an age-related change in Phase II metabolism. However, age-related changes in diet and polypharmacy result in many opportunities for drug interactions(169). In our study, subject were not permitted to take strong inhibitors or inducers of CYP2D6 or CYP3A4 within 28 days of study entry. Therefore, the 30% greater exposure to ODM in the elderly is not due to induction of CYP3A4 , the Phase I enzyme responsible for metabolism of (+)-ODM. This does raise the concern that elderly subjects may be at greater risk for side effects associated with greater (+)-ODM exposure due to CYP3A4 inhibition by medications such as such as macrolide antibiotics, azole antifungals and protease inhibitors. Conversely, they could be at greater risk for reduced opioid efficacy when tramadol is used with CYP3A4 inducers such as carbamazepine, Hypericum perforatum (St. John's Wort), phenobarbital, phenytoin and rifampin.

Furthermore, opioid-related efficacy and side effects of tramadol, among them sedation, are primarily linked to (+)-ODM (168) and this could be aggravated in patients with CYP2D6 extensive and super extensive metaboliser polymorphisms (170) or elderly subjects with greater renal impairment and frailty. The development of models such as our tramadol population PK model and our (+)-ODM PK/PD model permits the identification of covariates and patterns of covariates that affect the PK and PD of medicines across the human lifespan. Additional research and modeling of the effects of co-medication with inducers and inhibitors of CYP3A4 and tramadol and the effects of these in combination with CYP2D6

polymorphisms could be important future work in this area. This and other knowledge about the characteristics of elderly patients that pre-dispose them to side effects or lack of efficacy can subsequently be integrated in popPK predictive models to facilitate clinical research and improve pharmacotherapy. This approach has proven to be very successful for other special populations, like children or pregnant women and should also drive research on geriatric pharmacotherapy [2] where such knowledge is lacking.

As we conducted the analyses we identified several limitations and opportunities to improve the study and analyses. During the popPK analysis, we identified and excluded a subpopulation of 5 individuals exhibiting a food effect, since the majority of these subjects were young and showed rapid peak in plasma concentration and shorter half-life, including them may have implied a greater age related difference in the PK of tramadol; whereas this difference was related to the formulation not to the active moiety tramadol. It may have been better to include elderly patients with greater renal impairment and to have some patients with hepatic impairment. We had restricted the population to those with mild renal and hepatic impairment; all subjects but one had mild renal impairment and none had hepatic impairment. Therefore, we included a relatively healthy elderly population and excluded subjects who would be representative of frail elderly. The inclusion of frail elderly would be more representative of the population that is at higher risk to experience age related differences in PK/PD which could lead to altered efficacy and tramadol related side effects (10). That being acknowledged, it was extremely difficult to recruit healthy subjects over 75 to participate. One would expect that there would be fewer frail elderly who would be willing to participate. This research represents an important first step in understanding the PK of tramadol and ODM in the elderly. Future research opportunities include studying elderly patients who have greater frailty, including greater renal and hepatic impairment and patients with dementia. The data collected and PK and PK/PD models developed here could be utilised to support modeling of sparse data in these populations that could be difficult to recruit.

***Pain model***

The use of experimental pain models in human subjects can be a useful way to study analgesic response without many of the confounding factors often seen in large scale clinical trials (62). Understanding the PK and PD of tramadol was an important aspect of selecting an appropriate pain model. Tramadol has both a classic opiate mechanism of action where by it binds to μ-opioid receptors and it also enhances the release and inhibits the reuptake of 5-HT and NE in the descending pain pathways. Knowing that the opioid effect is primarily mediated by the (+)-ODM enantiomer and that the enhancement of the release and inhibition of the reuptake of serotonin are enantioselective effects of the tramadol; we did enantioselective analyses of the samples. We did not know at that time whether the PK of the enantiomers of the parent and ODM metabolite would be different in young and elderly subjects.

Aδ nociceptors respond to mechanical and thermal nociceptive stimuli while C nociceptors are respond to mechanical, thermal and chemical stimuli and opioid receptors modulate the activity of both of these types of fibres. Some C-fibres detect single sensations such as pinch or heat but most are polymodal. We examined a variety of pain models, such as thermal electrical and mechanical pain models, to select the model which was most likely to be able to detect the opioid action of tramadol. In the end we selected an electrical model based on literature findings that current sensory testing was more responsive than heat stimuli for opioid analgesics (31, 116, 120). Furthermore, in addition to being less likely to result in injury or lesion to the subject, because electrical stimuli directly stimulate the nerve fibres rather than the free nerve endings, it avoids the effect of variations in skin thickness and temperature and bone conductance that can confound thermal and vibratory sensory testing apparatuses (119). Although this carries the limitation that if there were an age-related difference in the sensitivity of free-nerve endings, an electrical model would not detect that. The device we selected, the Neurometer® is able to selectively stimulate Aδ and C fibres, the intensity of the stimulus is carefully controlled ensuring the same intensity of stimulus from one occasion to another on repeated measures within subject (as we wished to do with our study) and between subjects.

Results obtained from an experimental pain model conducted in volunteers may have limited generalisability to analgesic treatment of elderly patients. However, Olesen et al. (62) suggest that experimental pain models offer the opportunity to study pain responses when they are not blurred by other symptoms and where confounding environmental circumstances are as controlled as possible (62). Many later phase clinical trials, especially in analgesics produce inconclusive or negative results; experimental pain models offer the opportunity to understand the PD of analgesics on a smaller and more controlled scale. Population PK/PD analyses of these earlier research data offer the opportunity to optimise dosing regimens and design phase III clinical trials that take into account what is learned.

***Adequacy of the EPSM to detect a difference***

In planning the PD aspects of the trial conduct, we reviewed literature and identified that an ESPM was the most appropriate to assess the opioid effects of tramadol and ODM. Since the formulation in the study was a once-daily formulation it seemed most appropriate to assess PTT over more than 24 hours to characterise the entire effect curve. The design of the study was innovative in this aspect, with the repeated evaluations at 17 time points using two frequencies, throughout the administration interval and up to 30 hours after dosing. In planning the study procedures, we conducted a small volunteer study to better understand the kind and intensity of painful sensation caused by the Neurometer® and the two frequencies of electrical stimulation and refine the procedures for conducting the electrical stimulation procedures. In this volunteer study, we considered the use of the shin or the finger two sites that had been validated by the developer of the Neurometer®. We selected the non-dominant finger since that site provided easier access given the repeated sampling schedule. Furthermore, based on this study and review of the Neurometer® literature we left a minimum of 10 minutes, usually 20 minutes between stimulation to avoid hyperalgesia and temporal summation of C-fibres. The 250 Hz test which stimulates Aδ-fibres was conducted first, also to avoid any effect of C-fibre stimulation on the Aδ-fibres.

Having conducted the PK analyses and identified an age related difference in the active metabolite, (+)-ODM, we wanted to better understand how this affected the pain tolerance threshold of elderly subjects. In order to prepare to conduct this population PK/PD analysis we wanted to determine whether the EPSM, using the 5 or 250 Hz frequency, was able to capture changes in tolerance to pain intensity using PTT after the administration of a weak opioid in healthy volunteers. Furthermore, we wanted to determine whether there was an age related difference in response between elderly and young subjects and select the most appropriate level of stimulus (5 Hz or 250Hz) for the PK/PD analysis.

We used an AUEC analysis to determine this. AUEC is an appropriate approach as it takes into account the time course of response and the order in which data were obtained and the total analgesic effect will be composed of the sum of the true effect of the medication and the placebo effect (138).

Because the data can be complicated by the presence of both a non-zero baseline effect and a placebo effect, establishing baseline was an important aspect. We had originally intended to utilize baseline estimated from time zero, as we did training with the subjects the night before and expected to have consistent responses in the morning before dosing. In fact, the responses were highly variable sometimes for several hours after administration of the first dose and were often different between the two periods of study participation. Subjects attended clinic twice and were in a double-blind fashion administered active or placebo tablets at one or the other. Based on the approach recommended by Scheff et al. (138), we utilized estimation from $t_0$ and last evaluation for the AUEC analysis. Another option, since we had a placebo control, would have been the estimation from a separate control group or condition (placebo administration). We used the $t_0$ to last evaluation for the AUEC since we wanted to examine the placebo data on its own. In the (+)-ODM PK/PD analysis, we used data from the placebo control as it is possible to incorporate the placebo response into the model.

Studies in humans, in general, have drawn inconsistent conclusions with regard to the purported increase in pain perception and the decrease in pain tolerance in the elderly (22). Our exploratory results for pain tolerance showed a trend for baseline PTT to be lower in elderly than in the young. PD data in the young group after active administration failed to demonstrate significance against placebo in any of the analyses except for change in maximum pain tolerance ($\Delta$ $E_{max}$) after 250 Hz stimulation. This result could be a statistical anomaly given the variability of the data in the young. The point estimates for the mean $AUEC_{above}$ and $AUEC_{net}$ were consistently higher in the elderly during active administration phase for both 5 Hz and 250 Hz stimulations.

During the conduct of this analysis we identified additional limitations and opportunities to improve the research. For most measures, variability is higher in the young group with both the IQR (25% and 75%) and the $10^{th}$ and $90^{th}$ percentile error bars usually being greater. This greater variability could be a result of some younger subjects testing whether their pain tolerance would be higher than the cut-off limit of the Neurometer® apparatus. Although we did explain to the subjects the importance of using their own cut off rather than trying to test the machine, one way to improve the research would be to emphasise this point more clearly and explain the risk to the data of 'testing' the machine's limits. Since the objective of the EPSM is to demonstrate changes in pain tolerance and not the maximum tolerance of a given individual, anchoring the individual's maximum tolerance during test runs to a specific rating on a visual analogue scale could have helped the subjects more consistently discern and reproducibly identify the changes or lack thereof in their pain tolerance, thereby further reducing variability. Another approach for improvement would be to include an older 'young' group, such as 30-65 and a 'young old 'group of 65-75 years and 'old old' group of 75 years and older. This would have permitted us to analyse the data as continuous rather than categorical data and may have helped reduce variability.

To our knowledge this is the first study comparing the response in elderly to 5 Hz and 250 Hz stimulation. We found that the EPSM is able to detect a difference between placebo

and active administration phases for pain tolerance threshold in the elderly. Although both 5 Hz and 250 Hz can detect a difference, the effect size for 5 Hz is larger and seems more precise and reliable particularly in the elderly. This could be because the sensation caused by the 5 Hz stimulation is more unpleasant and therefore easier to recognize consistently.

When analyses were conducted to take into account the age related differences in pain tolerance, there were no significant differences in $E_0$ with 5 Hz or 250 Hz stimulation. A plausible reason for the fact that only elderly subjects showed a consistent and sustained increase in PTT during the active phase was identified in our previous noncompartmental PK analysis where a 30% higher exposure to (+)-0-Desmethyltramadol (+-ODM) was observed in elderly patients (171). As this metabolite is associated with much of the opioid analgesic effect of tramadol, this would roughly correspond to the 30% higher $AUEC_{above}$ and $AUEC_{net}$ observed in the elderly compared to young during the active period. Once again this pointed to the need to conduct further analysis of the PK/PD of (+)-ODM in the elderly.

Tramadol is a weak opioid and approximately 4000-fold less affinity for the μ-opioid receptor. (+)-ODM has 200 times the affinity of the parent for the μ-opioid receptor, however still much less than morphine (72). An important consideration, related to this, is that the dose of tramadol given in the study was in the middle of the dosing range (100-400 mg daily); this could have been insufficient in some subjects to provide a clear increase in pain tolerance.

***Age related differences in the PK/PD of (+)-ODM using the PTT as a biomarker for analgesic effect.***

The objective of this last analysis was to characterise (+)-ODM pharmacokinetics and pharmacodynamics using pain tolerance threshold from an electrically stimulated pain model to examine the effect of age in elderly and young subjects after single dose administration of tramadol 200 mg extended-release tablets. This work holds great importance as it represents the first PK/PD model of tramadol in elderly subject 75 years and older. It supports the suggestion of our early work that, in relatively healthy elderly volunteers, renal impairment

may occur earlier than hepatic impairment resulting in the potential for accumulation of (+)-ODM as a result of reduced renal clearance of the metabolite. It adds to that work by giving a clearer picture of the disposition and elimination of (+)-ODM in the elderly and that the increased (+)-ODM may in fact link to a 15% higher maximum treatment-related effect on pain tolerance threshold in the elderly versus young. Maximum plasma concentrations of (+)-ODM occurred later and plasma concentrations declined more slowly in the elderly than in young subjects. In the elderly, $V/f_m$ was 76% larger and $CL/f_m$ 16% slower.

At baseline ($E_0$), after taking into account a difference in $E_0$ between the periods, pain tolerance was similar between young and elderly subjects. As stated earlier, patients came to the clinic on two occasions, the mean E0 in period 1 was lower and less variable in subjects on the first occasion. This could be due to the subjects being less anxious, more comfortable or more bored with the pain stimulus procedures and apparatus on the second occasion leading to less care in selecting the PTT. It could also be due to a reduced sensitivity to the electrical stimuli, however, since there was a 7 day washout between the clinic occasions and we inspected the stimulation site each time to be sure that there was no lesion or damage and we did not find any, it seems unlikely that this is the source. Also, if sensitivity was impacted we would expect to see a period effect on other parameters such as $E_{max}$ and $C_{50}$. The model was able to account for the effect of period on baseline. There was no effect of treatment sequence on baseline or other parameters.

The $E_{max}$ parameter was 2.5 times higher in the elderly. While we saw a 15% higher maximum treatment-related effect in the elderly, we did not see any difference in (+)-ODM $C_{50}$ compared to young subjects. This likely reflects the higher exposure to the active metabolite because of reduced renal clearance. In our study $C_{50}$ reflects sensitivity to pain tolerance for (+)-ODM. Given that the general consensus, supported by our findings, is that PTT to electrical stimulus remains unchanged with age, we do not expect that the $C_{50}$ would be different in the elderly compared to the young. A change in $C_{50}$ would suggest that, at the cellular level, receptor binding and associated response are different. We saw no evidence that,

with regard to PTT, there is a change in sensitivity to (+)-ODM. However, we did not determine the extent of (+)-ODM plasma protein binding in both groups. It is therefore difficult to have a definite answer until changes in the free fraction, the active moiety, has been ruled out.

The population PK/PD model that best fit the data, modelled placebo as a linear, time-independent function. We expected that a placebo model that was time dependent would have better fit the data. In exploring this, we discovered that the overall response in placebo period appeared stable across the whole time interval of the PD assessments although placebo responses at particular times during the evaluation period may have been greater or less than the baseline response. We attempted modeling the placebo response independently but the model was over-parametrised and did not converge.

The work in my thesis is a beginning in understanding the analgesic response to tramadol and O-desmethyltramadol in the elderly. But many questions remain. A clearer understanding of elderly patients' individual characteristics and how they affect the PK and PD of tramadol, particularly in those who have characteristics associated with frailty is an important are for further exploration. Conducting a pharmacokinetic study similar to ours in patients who demonstrate the increased vulnerability resulting from aging-associated decline in reserve and function across multiple physiologic systems, defined as frailty (13), is important to a better understanding of why these patients are at greater risk for many tramadol side effects, amongst them seizures. This in turn can help us determine how we can mitigate these risks to the greatest degree possible. Important future questions include:

- What, if any, age-related changes in PK are present in subjects with greater renal impairment?
- What is the effect of hepatic impairment on PK of tramadol in the elderly, especially given the increased exposure to (+)-O-desmethyltramadol seen in elderly subject in our study?

- What would the effect of unintended weight loss, a phenotypic characteristic of frailty have on the Vd of tramadol?
- Do any of these PK changes explain the greater occurrence of seizures and possibly hypoglycemia seen in elderly subjects?
- Could inhibition by co-medications of CYP3A4 mediated metabolism or glucuronidation of O-desmethyltramadol result in higher exposure of elderly subjects to the active metabolite and what are the effects of that, especially in more frail elderly who may have greater renal impairment?

In addition, further exploration of the pain tolerance of elderly subjects under both active treatment and placebo, can help to understand the changes in nociceptive processes as aging occurs. Comparison to younger subjects could perhaps elucidate why we were able to detect a difference in elderly subjects PTT when administered active versus placebo but not in young subjects. This could be revelatory of sources of variability in PTT and in placebo response in young and elderly subjects which could help in designing better Phase 3 studies in pain and developing better medications for patients in pain.

The results of this research program indicate that there are differences in the PK/PD of tramadol in elderly subjects as compared with young. In particular, 30% greater exposure to (+)-ODM raises the concern that accumulation could occur, although based on this data the accumulation factor is low (1.1). Suggesting that dose adjustment in relatively healthy elderly patients with mild renal impairment is likely unnecessary. In young subjects, after hepatic biotransformation, tramadol and its metabolites are largely eliminated by the kidneys (~90%) with the remainder eliminated in feces. Approximately 12-25% of an oral dose of tramadol is excreted unchanged in urine and 15% as ODM and the rest is other metabolites (101, 102). In its guidance on pharmacokinetics in patients with impaired renal function, the U.S. Food and Drug Administration recommends that dose adjustment should occur in patients with renal insufficiency in medications that are excreted more than 50% unchanged (172). This is not the case with tramadol, so it is unlikely that purely on that basis, dose adjustment would be recommended. However, the trend we observed suggests that an awareness of the renal status

of elderly patients taking tramadol is important and that careful consideration should be given to using tramadol in patients with the potential for greater levels of renal impairment, the potential for hepatic impairment and frailty. Furthermore, there is a strong association between the increased exposure and increased pain tolerance threshold ($E_{max}$). Literature indicates that the μ-opioid activity of tramadol is primarily related to (+)-ODM, μ-opioid binding is associated with analgesia but it is also associated with a variety of side effects including nausea/vomiting, dizziness, drowsiness, tiredness, sweating and dry mouth, all of which can cause concerns for elderly patients, especially frail elders. In the U.S. in 2011, 35% of emergency room (ER) visits involving adverse reactions to tramadol were undertaken by older adults (aged 65 and older) (173). Of all tramadol related adverse events that resulted in an ER visit 17% resulted in hospitalisation and half of those were in adults 65 years or older, it is important to weigh the potential for clinical utility in the elderly against the risks understanding the difference in the PK and PD of tramadol and its active metabolite, (+/-)-O-desmethyltramadol is vital.

## 7.2 Conclusions

Our research found that tramadol, a mild opioid analgesic, and its active metabolite (+)-ODM, demonstrate altered pharmacokinetics and pharmacodynamics in elderly subjects:

- While tramadol exposure ($C_{max}$ and AUC ) between age-groups was similar, there were significant differences in $V_{area}/F$ (mean 34% higher) and $k_{el}$ (mean 28% lower) in the elderly.
- More significantly, the exposure to ODM was 35% greater in the elderly and both renal clearance and overall elimination were slower resulting in a 50% longer mean elimination half-life in the elderly.
- Using a ESPM that was adequate to detect a difference in pain tolerance threshold, we determined that, while $E_0$ and $C_{50}$ was similar in young and elderly subjects, the $E_{max}$ parameter is 2.5 times higher and maximum treatment related effect is 15% higher in the elderly

This is the first research program to report as extensively on the PK and PD of tramadol in the elderly. The value of the research program goes beyond that of a better understanding of the PK of tramadol, to add value in our understanding of the relative contribution of hepatic and renal insufficiency to age related alterations in the PK of tramadol in generally healthy elderly people and can therefore contribute to the development of population models to support further research in medicines in the elderly. Furthermore, it is the first population PK/PD model of (+)-ODM in subjects 75 and older. Our findings show that age related changes in hepatic and renal clearance can result in proportional ODM accumulation, and may explain increased sensitivity to (side) effects in the elderly. This is of clinical significance since opioid-related efficacy and side effects of tramadol, among them sedation, are primarily linked to (+)-ODM.

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# Appendix 1: Concomitant medications during the study

All medications taken by subjects during the course of the study were recorded. Subjects who could not follow the requirements for concomitant medication use listed below could not participate in the trial, with the exception that elderly subjects (age ≥ 75 years) were allowed certain chronic medications (if deemed acceptable by the study physician) and the dose was stable:

- Subjects who had used any drugs or substances known to be strong inhibitors of CYP enzymes within 10 days prior to the first dose. Examples of strong CYP3A4 inhibitors include: protease inhibitors (ritonavir, indinavir), some macrolide antibiotics (clarithromycin, telithromycin), some azole antifungals (itraconazole, ketoconazole, nefazodone) while examples of strong CYP2D6 inhibitors include: certain SSRIs (fluoxetine, paroxetine), buproprion and quinidine.
- Subjects who had used any drugs or substances known to be strong inducers of CYP enzymes within 28 days prior to the first dose. Examples of strong CYP3A4 inducers include Carbamazepine, Hypericum perforatum (St. John's wort), phenobarbital, phenytoin and rifampin and a strong inducer of CYP2D6 is a hypnotic sedative called glutethimide.
- Subjects who had received monoamine oxidase inhibitors (MAOI) or antidepressants (tricyclic or SSRIs), within 28 days prior to the first dose due to the risk of serotonin syndrome with tramadol.
- Subjects who had received drugs belonging to the opioids/analgesic class, within 5 elimination half-lives prior to the first dose, in order to ensure that tramadol was the only analgesic in the subjects' circulation during the study,
- Subjects who had received coumarin derivatives (e.g warfarin) or digoxin, within 28 days prior to the first dose, to avoid excessive bleeding or bruising due to the extensive blood sampling schedule.
- Subjects who had received CNS depressant drugs (such as benzodiazepines, barbiturates, sedative H1 antihistamines, neuroleptics, some beta-blockers,

anxiolytics other than benzodiazepines), tricyclic compounds (such as cyclobenzaprine, promethazine), drugs increasing serotonin levels or thalidomide within 5 elimination half-lives prior to the first dose.

- Females of childbearing potential were permitted to enter the study if they were taking contraceptives as long as they had been used for at least 3 months prior to the first dose of the study.

If drug therapy other than that specified in the protocol was required, a decision to continue or discontinue the subject was to be made, based on the time the medication was administered and its pharmacology and pharmacokinetics. No prohibited medications were administered during the study conduct.