UNIVERSITI PUTRA MALAYSIA

POST-SURGICAL ANALGESIC PROPERTIES AND PHARMACOKINETICS OF TRAMADOL IN DOGS UNDERGOING ELECTIVE SURGERY

SALISU BUHARI

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By

SALISU BUHARI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To every healthy mind. A mind which puts truth to the test and knows it from the wreck of the wrong. A mind which weighs all that has been said in the scale of justice, and always comes out in favor of reason. A mind which compares words and the saying, and has the ability to distinguish between the logical and not so logical and between the strong and the feeble.

(Anonymous)

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairperson: Kalthum Hashim, PhD

Faculty: Veterinary Medicine

Post-surgical pain is a distressing phenomenon associated with potential tissue damage in dogs. Many pain relief options such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioid receptor agonists range widely in both their therapeutic and side effects. Atypical opioids such as tramadol are used in veterinary practice as an alternative due to their dual mechanism of action and have been designed to overcome the side effects through an opiate-sparing effect. Despite the wide clinical applications of tramadol in dogs, its analgesic efficacy following subcutaneous administration, renal and hepatic safety following repeated administration during surgery and the effect of surgery as well as gender on its pharmacokinetics, are not well documented. Thus, this study was conducted to compare the effect of routes of administration of tramadol on post-surgical pain management in dogs, the effects of single and second dose of tramadol administration on renal and hepatic changes in dogs undergoing surgery and the effects of surgery on the pharmacokinetics of tramadol following subcutaneous and intravenous administration in male and female dogs. It was therefore hypothesized that subcutaneous administration of tramadol can

provide equivalent analgesia when given intravenously route of administration, and that first and second dose of tramadol can provide post-surgical analgesia without pathological effects. Also, surgery and gender differences can influence the pharmacokinetic parameters of tramadol in dogs.

A ‘double-blind’ trial was carried out comparing the analgesic efficacies of tramadol (3 mg/kg) given subcutaneously (SC) with intravenous (IV) administration following ovariohysterectomy. Eighteen female dogs were divided into 3 groups of six in each group. Tramadol (3 mg/kg) was given to Group 1 subcutaneously and to Group 2 intravenously, Group 3 was a negative control (without tramadol). A significant increase in pain perception was observed in group 3, while groups 1 and 2 had an equal postoperative analgesic activity indicated by the analgesiometer test, serum interleukin 6(IL-6) and serum beta-endorphins (β-end) physiological parameters. The measurement of serum interleukin 6 (IL-6) and serum beta-endorphins (β-end) correlated with post-surgical pain stimulation in all groups. A higher relationship was observed between analgesiometer values and β-end.

Twenty-five female dogs, five in each group, were used to study the effect of single and double doses of tramadol on hepatic and renal changes and functions. Groups 1 and 2 received 3 mg/kg of tramadol by subcutaneous and intravenous injections, respectively, during premedication. Groups 3 and 4 received similar doses of tramadol during premedication, which was repeated 2 hours after the initial dose through subcutaneous and intravenous injections, respectively. Group 5 served as a negative control. Blood samples (2 mL) were taken at 0, 2 and 4 hr after tramadol administration while liver and kidney biopsies were taken before the end of surgery

5 hours after first tramadol administration. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly lower in groups 2, 3 and 4 when compared to group 5 at 4 hours post-tramadol administration. Significant increases in AST were observed in groups 1, 3 and 5 at 2 hours and at 4 hours in groups 1 and 5. Histopathological changes in the liver and kidney included congestion, edema and cellular infiltration which occurred less frequently in groups 3 and 4. The changes suggested side effects of pentobartone anaesthesia and not tramadol.

Thirty-six dogs comprising twenty-four females and twelve males were used in studying the influence of surgery and gender-differences on tramadol pharmacokinetics. The dogs were grouped into six equal groups of six dogs each viz: groups 1 and 2 were female dogs and received 3 mg/kg tramadol via the intravenous and subcutaneous route, respectively, and both underwent surgery; groups 3 and 4 were also female dogs and received similar doses of tramadol via the intravenous and subcutaneous route, respectively, without surgery, while groups 5 and 6 were male dogs without surgery and were given tramadol via the intravenous and subcutaneous route, respectively.

The outcome of this study showed that surgery significantly affected the biotransformation process of tramadol, as indicated by a 2-fold increase in its elimination half-life (1.10 ± 0.18 hr) in groups 1 and 2 compared to groups 3 and 4 (0.49 ± 0.07 hr). Serum tramadol concentration was significantly higher in groups 1 and 2 (770.21 ± 117.76 ng/ml) compared to groups 3 and 4 (117.61 ± 85.16 ng/ml) which was reflected by a significantly lower clearance value (3.98 ± 0.56 ml/min/kg)

in groups 1 and 2 compared with groups 3 and 4 (21.06 ± 9.34 ml/kg). The results suggested that surgery and anaesthesia have an effect on both the distribution and elimination of tramadol in dogs.

A significantly shorter duration to reach high plasma tramadol concentration (0.17±0.01 hr) in male dogs compared with (0.75±0.01 hr) female dogs was observed. The rate of movement of the tramadol from the first compartment to the second compartment was significantly ($p < 0.05$) slower (5.99±4.1 l/hr) in males, compared with 13.34±12.58 l/hr found in female dogs. Similarly, higher ($p < 0.05$) systemic bioavailability (29.65±11.7%) among males was observed compared to females (15.68±4.14%), suggesting a faster passage of the drug from blood to the organs in female dogs. There was no significant difference in the plasma concentration, distribution half life, elimination half life and clearance was observed between intravenous and subcutaneous routes of tramadol administration.

This study concluded that: the subcutaneous route of administration of tramadol was similar to the intravenous route in the management of post-surgical pain in dogs. Also, an additional dose of tramadol at 3 mg/kg administered intravenously or subcutaneously is safe at 2 hours interval during surgery without causing hepatic and renal damage in dogs. Finally, after subcutaneous administration of tramadol in dogs, the drug was rapidly absorbed into the blood stream while the bioavailability was very low due to surgery and anaesthesia. These findings suggested that in the dog, the subcutaneous pharmacokinetics of tramadol is similar to the intravenous and both routes are influenced by surgery and anaesthesia. In addition, male dogs require a higher frequency of tramadol administration due to a faster biotransformation,

hence the concentration profile supports an effective surgical and clinical duration of three hours in female dogs.

CIRI-CIRI ANALGESIK SELEPAS PEMBEDAHAN DAN FARMAKOKINETIK TRAMADOL PADA ANJING YANG MENJALANI PEMBEDAHAN ELEKTIF

OLEH

SALISU BUHARI

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Pengerusi: Kalthum Hashim, PhD

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Kesakitan selepas pembedahan adalah satu fenomena yang dikaitkan dengan potensi kerosakan tisu pada anjing. Beberapa opsyen rawatan untuk mengurangkan kesakitan seperti penggunaan secara meluas drug steroid nyah inflamasi (NSAID) dan reseptor agonis opioid keatas kedua kesan terapeutik dan sampinganya. Opioid tidak lazim seperti tramadol telah digunakan dalam amalan veterinar sebagai alternatif kerana dwi mekanisme tindakannya dan telah dibentuk supaya dapat mengatasi kesan sampingan melalui kesan lawanan opoids. Walaupun aplikasi klinikal tramadol telah dilakukan pada anjing dengan meluas, keberkesanan tahan sakit selepas rawatan subkutaneus, kesihatan buah pinggang dan hati berikutan rawatan yang berulang semasa pembedahan dan kesan pembedahan serta fakta jantina keatas farmakokinetik tidak didokumenkan dengan baik. Oleh itu, kajian ini telah dijalankan untuk membandingkan kesan rawatan tramadol dalam mengawal kesakitan selepas pembedahan pada anjing, kesan dos pertama dan kedua rawatan tramadol terhadap perubahan pada buah pinggang dan hati pada anjing yang menjalani pembedahan dan kesan pembedahan pada farmakokinetik tramadol selepas rawatan subkutaneus dan intravena pada anjing jantan dan betina. Oleh itu telah dihipotesiskan bahawa tramadol diberi secara subkutaneus boleh menghasilkan

analgesia setanding dengan pemberian secara intravena, dan bahawa dos yang pertama dan kedua tramadol boleh menghasilkan analgesia selepas pembedahan tanpa kesan sampingan patologi. Begitu juga, pembedahan dan perbezaan jantina boleh mempengaruhi parameter farmakokinetik tramadol pada anjing.

Percubaan 'double-blind' telah dijalankan untuk membandingkan keberkesanan rawatan analgesik tramadol (3mg/kg) yang diberi secara subkutaneus (SC) dengan yang diberi secara intravena (IV) selepas ovariohisterektomi. Lapan belas anjing betina telah dibahagi kepada tiga kumpulan, setiap satu mempunyai enam ekor. Tramadol (3 mg/kg) diberi kepada Kumpulan 1 secara subkutaneus dan kepada Kumpulan 2 secara intravena, Kumpulan 3 adalah kawalan negatif (tanpa tramadol). Satu peningkatan yang ketara dalam persepsi kesakitan didapati dalam kumpulan 3, manakala kumpulan 1 dan 2 mempunyai aktiviti analgesik pasca pembedahan seperti yang pamerkan oleh ujian analgesiometer, interleukin serum 6 (IL-6) dan serum beta-endorfin (β-end) parameter fisiologi . Pengukuran interleukin serum 6 (IL-6) dan serum beta-endorfin (β-end) dikaitkan dengan rangsangan kesakitan pasca pembedahan dalam semua kumpulan. Satu pertalian yang lebih tinggi telah diperhatikan antara nilai analgesiometer dan β-end.

Dua puluh lima anjing betina, lima dalam setiap kumpulan, telah digunakan untuk mengkaji kesan dos tunggal dan berganda tramadol terhadap perubahan dan fungsi pada hati dan buah pinggang. Kumpulan 1 dan 2 yang menerima tramadol 3 mg/kg secara subkutaneus dan intravena semasa para-rawatan. Kumpulan 3 dan 4 yang menerima dos tramadol yang sama semasa para-rawatan dan diulang selepas 2 jam secara subkutaneus dan intravena. Kumpulan 5 yang bertindak sebagai kawalan

negatif. Sampel darah (2 mL) telah diambil pada 0, 2 dan 4 jam selepas rawatan tramadol manakala biopsi hati dan buah pinggang telah diambil 5 jam sebelum pembedahan berakhir, iaitu selepas rawatan tramadol yang pertama. Tahap aminotransferase alanin (ALT) dan aspartate aminotransferase (AST) didapati lebih rendah dalam kumpulan 2, 3 dan 4 apabila dibandingkan dengan kumpulan 5 pada 4 jam selepas rawatan tramadol. Peningkatan yang ketara AST telah diperhatikan dalam kumpulan 1, 3 dan 5 pada 2 jam dan pada 4 jam dalam kumpulan 1 dan 5. Perubahan histopathologi pada hati dan buah pinggang adalah termasuk kongesi, edema dan penyusupan selular yang kurang dalam kumpulan 3 dan 4. Perubahan berkemungkinan disebabkan oleh kesan sampingan pentobartone dan bukan disebabkan oleh tramadol.

Tiga puluh enam anjing yang terdiri daripada 24 betina dan 12 jantan telah digunakan untuk mengkaji pengaruh pembedahan dan perbezaan jantina pada farmakokinetik tramadol. Anjing telah bahagikan kedalam enam kumpulan yang sama, iaitu masing-masing mengandungi enam ekor anjing: kumpulan 1 dan 2 anjing betina telah menerima tramadol 3 mg/kg secara intravena dan subkutaneus dan menjalani pembedahan. Kumpulan 3 dan 4 juga anjing betina menerima dos tramadol yang sama secara intravena dan subkutaneus tanpa pembedahan, manakala kumpulan 5 dan 6 ialah anjing jantan tanpa pembedahan dan diberi tramadol secara intravena dan subkutaneus.

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Hasil kajian ini menunjukkan pembedahan mempengaruhi proses biotransformasi tramadol, seperti yang ditunjukkan melalui peningkatan 2 kali ganda dalam penghapusan separuh hayat (1.10 ± 0.18 jam) pada kumpulan 1 dan 2 berbanding

dengan kumpulan 3 dan 4 (0.49 ± 0,07 jam). Kepekatan serum tramadol adalah jauh lebih tinggi dalam kumpulan 1 dan 2 (770,21 ± 117,76 ng / ml) berbanding kumpulan 3 dan 4 (117,61 ± 85,16 ng / ml), yang ditunjukkan oleh nilai perkumuhan yang jauh lebih rendah dengan anggaran (3.98 ± 0,56 ml / min / kg) dalam kumpulan 1 dan 2 berbanding dengan kumpulan 3 dan 4 (21,06 ± 9,34 ml / kg). Keputusan mencadangkan bahawa pembedahan dan anestesia memberi kesan terhadap pengedaran dan perkumuhan tramadol pada anjing.

Satu tempoh yang lebih pendek untuk mencapai kepekatan plasma tramadol yang tinggi (0.17 ± 0.01 jam) pada anjing jantan berbanding (0,75 ± 0.01 jam) anjing betina. Kadar pergerakan tramadol dari kompatmen pertama ke kompatmen kedua adalah ketara ($p < 0.05$) perlahan (5.99 ± 4.1 l / jam) bagi anjing jantan, berbanding dengan 13.34 ± 12.58 l / jam pada anjing betina. Begitu juga, didapati sistemik bioavailabiliti lebih tinggi ($p < 0.05$) (29.65 ± 11.7%) di kalangan anjing jantan berbanding dengan anjing betina (15.68 ± 4.14%). Ini menunjukkan bahawa laluan dadah dari darah kepada organ adalah lebih cepat pada anjing betina. Tiada perbezaan ketara pada rawatan tramadol yang diberikan secara intravena dan subkutaneus .

Kajian ini boleh disimpulkan bahawa tiada perbezaan didapati pada rawatan tramadol secara subkutaneus atau intravena dalam mengawal kesakitan selepas pembedahan pada anjing. Juga, dos tambahan tramadol 3 mg / kg yang diberi secara intravena atau subkutaneous adalah selamat pada selang 2 jam semasa pembedahan tanpa menyebabkan kerosakan hati dan buah pinggang. Akhirnya, selepas pemberian secara subkutaneus pada anjing, dadah pantas menyerap ke dalam saliran darah

manakala bioavailabiliti adalah sangat rendah disebabkan oleh pembedahan dan anestesia. Penemuan ini mencadangkan bahawa, farmakokinetik subkutaneus tramadol adalah serupa dengan intravena dan kedua-dua laluan dipengaruhi oleh pembedahan dan anestesia. Di samping itu, anjing jantan memerlukan rawatan tramadol yang lebih kerap disebabkan oleh biotransformasi yang lebih cepat, maka profil kepekatan menyokong tempoh keberkesanan pembedahan dan klinikal selama tiga jam pada anjing betina.

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APPROVAL SHEET

I certify that a Thesis Examination Committee has met on 6th September 2012 to conduct the final examination of Salisu Buhari on his Ph.D thesis entitled "**Post-Surgical Analgesic Properties and Pharmacokinetics of Tramadol in Dogs Undergoing Elective Surgery**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, or concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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SALISU BUHARI

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

-	Negative
%	Percentage
~	Approximately
+	Positive
µ	Mu
µl	Microliter
µm	Micrometer
µmol	Micromolar
^{0}C	degree centigrade
5HT	5-hydroxytryptamine (serotonin)
A	intercept of the distribution phase
ALT	alanine aminotransferase
ANOVA	Analysis of variance
AST	aspartate aminotransferase
AUC	area under the curve
$AUMC_{0-\infty}$	Area under the first moment curve from 0 to infinity
AUP	Animal Utility Protocol
B	intercept of the elimination phase
BICIN	N,N,-bis(2-hydroxylethyl)-glycine
BUN	blood urea nitrogen
C_0	Concentration at time 0
Ca^{++}	calcium ion
CAN	acetonitrile
CAPSO	3-(cyclohexylamino)-2-hydroxyl-1-propanesulfonic acid
Cl	body clearance

Cl_t	Total body clearance
C_{max}	Maximum concentration
CMPS	composite measure pain scale
CNS	central nervous system
Cp	Plasma concentration
CTRL	negative control
CYP	isoenzyme cytochrome
DDH_2O	deionised distilled water
DNA	Deoxyribonucleic Acid
ED	Extradural
ELISA	enzyme-linked immunosorbent assay
EtAc	ethylacetate
F (%)	Bioavailability
GLDH	Glutamate dehydrogenase
H & E	Hematoxylin & Eosine
H^+	hydrogen ion
HCl	Hydrochloride
HPLC	High performance liquid chromatography
Hr	Hour
IL-6	interleukin 6
IM	Intramuscular
IV	Intravenous
IV1X	single intravenous
IV2X	repeated intravenous;
K^+	potassium ion
k_{12}	Rate of movement from compartment 1 to compartment 2
k_{21}	Rate of movement from compartment 2 to compartment 1
Kg	Kilogram
LAAM	levo-alphaacetylmethadol hydrochloride

LC	liquid chromatography
LD50	lethal dose
LDH	lactate dehydrogenase
Ln	natural log
M1	*O*-desmethyltramadol
M2	N-desmethyl tramadol
M3	N, N-didesmethyl tramadol
M4	N, N, O-tridesmethl tramadol
M5	N, O-didesmethyl tramadol
MDH	malate dehydrogenase
Mg	Milligram
Min	Minute
Mm	Milimeter
MRT	Mean residence time
N	Newton
NA	Noradrenalin
NA	Not applicable
NADH	nicotinamide adenine dinucleotide hydrogenase
Ng	Nanogram
Nm	Nanometer
NMDA	*N*-methyl *D*-aspartate
NSAID	non steroidal anti-inflammatory drugs
O.D.	optical density
OHE	Ovariohysterectomy
PAG	periaqueductal grey
PAG	periaqueductal grey
PO	*per os*
POMC	proopiomelanocortin
RM-ANOVA	Repeated measure analysis of variance

RVM	rostral ventromedial medulla
RVM	rostral ventromedial medulla
SC	Subcutaneous
SC1X	single subcutaneous
SC2X	repeated subcutaneous
SD	Standard deviation
SP	Substance P (neuropeptide)
SPE	Solid phase extraction
STAT	signal transducers and activators of transcription
$t_{½}$ λz	Half-life of the terminal portion of the curve
t½α	distribution half-life;
t½β	elimination half-life
T_{max}	Time to maximum concentration
TNF	tumor necrosis factor
Uv	ultraviolent
V1	Volume of compartment 1
V2	Volume of compartment 2
Vd	volume of distribution
A	Alpha
B	Beta
β-end	Beta endorphin
Δ	Delta
K	Kappa
λz	First-order rate constant
$\mu g/m^2$	Microgram per meter square

CHAPTER 1

GENERAL INTRODUCTION

Post-surgical pain is a distressing phenomenon associated with potential tissue damage in dogs (Abu-Seida, 2012). Ovariohysterectomy (OHE) is the most common elective surgical procedure used for sterilization of female dogs in small animal practice. The procedure offers the benefits of population control and decreased risk of potentially life-threatening diseases such as mammary cancer and pyometra (Olson and Johnston, 1993). Ovariohysterectomy causes pain and morbidity from tissue trauma, organ manipulation, and postoperative inflammation (Davidson *et al*., 2004). Both veterinarians and pet owners are becoming increasingly concerned about postoperative pain and morbidity associated with most elective surgeries and this has resulted in increased interest in effective post-surgical pain management protocols (Lemke *et al*., 2002).

Historically, animals were thought to perceive pain differently than humans or not to feel pain following injury. As an inference to this concept, it was believed that pain following surgery or injury was beneficial to animals because it limited their activities and thus prevented further injury and stress (Viñuela-Fernández *et al.*, 2007). Today there is a better understanding of how pain develops and perpetuates (Rice *et al.*, 2008). It is now well established that animals and humans have similar neural pathways for the development, conduction, and modulation of pain (Viñuela-Fernández *et al.*, 2007). According to the principle of analogy, because cats and dogs have neural pathways and neurotransmitters that are similar, if not identical, to those of humans, it is highly likely that animals do experience pain in a similar manner.

Veterinary practitioners also have more insight into how most drugs work to modulate pain and how and why a therapeutic strategy can benefit patients. Uncontrolled surgical pain decreases quality of life in all patients, and prolongs recovery (Hellyer *et al*., 2007).

Recognition of pain in human is a subjective experience, but its nature in animals can be challenging since many animals have evolved to hide such signs of illness and pain (Souza *et al.*, 2008). However, the consensus amongst veterinarians and researchers is that alterations in animals' behaviour are likely to accompany pain. It has therefore been accepted that pain or distress that may result in an animal undergoing biomedical research should be prevented or minimized as far as possible (Roughan and Flecknell, 2003). Many pain relief options such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioid receptor agonists range widely in both their therapeutic and side effects (Giorgi, 2012). The clinical application of many available analgesics in veterinary practice is limited due to development of unwanted side effects, especially in chronic painful conditions (Bancos *et al.*, 2009; McMillan *et al*., 2008). Opioids such as pethidine have been shown to be an effective analgesic in the clinical setting in dogs (Waterman and Kalthum, 1989a), albeit with a short duration of action. Opioids also produce reversible behavioural (KuKanich *et al*., 2008) and physiological (Wagner, 2002) side effects in dogs. An atypical opioid such as tramadol is used in veterinary practice as an alternative, due to their dual mechanism of action and have been designed to overcome these side effects through an opiate-sparing effect (Giorgi, 2012).

Tramadol is an analgesic with a dual mechanism of action which has been found to bind to the μ_1-opioid receptor (Guedes *et al.,* 2005; Kukanich and Papich, 2004) as well as to inhibit the monaminergic pathway i.e., noradrenaline (NA) and serotonin (5HT) re-uptake (Kubota *et al.,* 2008; McMillan *et al.,* 2008). For this reason, tramadol is also referred to as an "atypical opioid" and is only partially inhibited by the opioid receptor antagonist naloxone (McMillan *et al.,* 2008).

In the last two decades, tramadol has been used clinically in the management of pain in humans following orthopaedic and gynaecological surgeries as well as in non-surgical conditions such as for renal, dental, abdominal, haematologic and neoplastic pain (Giorgi *et al.,* 2009; Tuncer *et al.,* 2003). In addition, tramadol has low abuse potential and minimal effects on cardiopulmonary and gastrointestinal motility (Kukanich and Papich, 2004; Scott and Perry, 2000).

The strategy of administering opioids and other groups of analgesics before surgery is referred to as *preemptive analgesia,* which is aimed at preventing central sensitization of nociception (Mastrocinque and Fantoni, 2003). Preemptive administration of tramadol has been shown to significantly reduce the amount of inhalant anaesthetic required for procedures in humans (Wordliczek *et al*., 2002) and dogs (Seddighi *et al.,* 2009). Pain control, the most beneficial aspect of preemptive analgesia, is a crucial factor for patient care. Uncontrolled pain in veterinary patients can result in unwanted complications, including cardiovascular stress, immunosuppression, anorexia and cachexia (Hancock *et al.,* 2005).

In recent years, interest in studying renal and hepatic function during anaesthesia and surgery in small animals has increased greatly (Kongara *et al*., 2009) due to their primary role in drug metabolism and excretion (Mercadante and Arcuri, 2004), which predisposes them to various degrees of injury in chronic administration (Atici *et al*., 2005). Tramadol is metabolized by an isoenzyme, cytochrome CYP-450 2D6 (CYP2D6) (KuKanich and Papich, 2004), to produce an active metabolite *O*-desmethyltramadol (M1). This is mediated by the hepatic canine ortholog of CYP2D15 (Tasaki *et al.,* 1998) before its rapid elimination by the kidneys (McMillan *et al.,* 2008). Due to the fact that tramadol has a rapid elimination half-life, more frequent dosage intervals are needed to potentiate effective pain control such as that caused by major surgeries (McMillan *et al.,* 2008; KuKanich and Papich, 2004). Despite the increased use of tramadol in the management of chronic and postoperative pain in animals, there is limited information on its effects on hepatic and renal functions following its repeated administration in prolonged surgical procedures in dogs. Many previous studies focused on physiological changes that occur during the surgery (Wunsch *et al*., 2010), which can be attributed to immediate problems associated with cardiopulmonary functions in most surgical procedures (Wunsch *et al.,* 2010; Valtolina *et al.,* 2009). Thus, to incorporate an appropriate analgesic protocol, it is essential that both laboratory and clinical investigations be taken into consideration. More importantly, the biotransformation of tramadol in dogs differs from other species (Vettorato *et al.,* 2010) and their sensitivity to NSAIDs is increased (Lascelles, 1999).

Unlike in humans, tramadol has recently gained significant attention in veterinary practices (McMillan *et al*., 2008). There is an increased interest in studying the

differences that exist between the metabolism of drugs in human and dogs, because while the use of dogs by pharmaceutical companies for preclinical drug testing is rampant (Lauren, 2006) there is still a lack of evidence that its metabolic pathways are similar to human's. In addition, more studies are needed on the pharmacokinetics and pharmacodynamic properties of tramadol in animals such as dogs, cats, horses and goats (Cagnardi *et al.,* 2011; Pypendop and Ilkiwi, 2008; Shilo *et al.,* 2008; Kukanich and Papich 2004). Using dogs, Kukanich and Papich (2004) studied the pharmacokinetic properties of intravenous (IV) and oral (PO) tramadol at 4.4 mg/kg and 100 mg per animal, respectively, as well as its M1 metabolite (1 mg/kg IV). Mastrocinque and Fantoni (2003) compared tramadol (2 mg/kg) with morphine (0.2 mg/kg) after IV injection for the control of post-operative pain following ovario-hysterectomy and found tramadol produced analgesia equivalent to a potent opioid like morphine.

Pharmacokinetics deals in part with the mathematical description of the time course of a drug's absorption, distribution and elimination by means of a suitable model (Rowland and Tozer, 1995). To facilitate the study of the pharmacokinetic behaviour of a drug, the body is depicted as a system made up of one, two or three distribution compartment models (Figure 1.1). If a drug distributes very rapidly relative to its rate of elimination, the drug's disposition within the body behaves as though the body is a single homogenous compartment, and is referred to as a one-compartment open model.

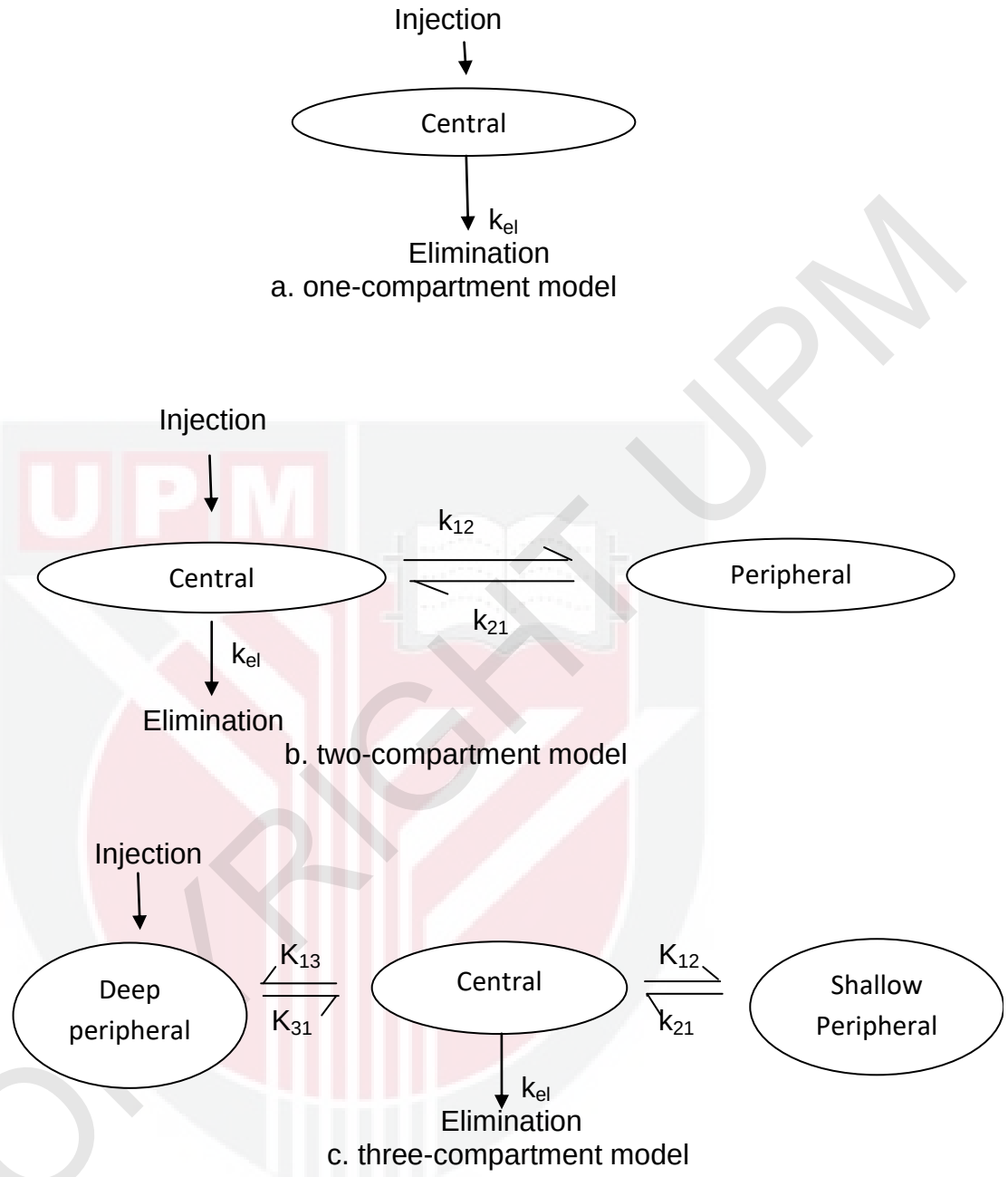

Figure 1.1 Diagrammatic representation of compartmental models used in the pharmacokinetic study (adapted from Kalthum, 1988).

A two-compartment model possesses an additional peripheral compartment corresponding to extravascular tissue in addition to the central plasma compartment (Rang, 2003). While in three and more than three-compartmental models (multi-compartment), drugs are distributed into deeper peripheral tissues and are not usually used in routine clinical practice. However, a non-compartment model appears more convenient and straight forward because it does not split the body into different compartments, but will demand more frequent blood sampling for an accurate area under the curve (AUC) calculation. The disposition of tramadol has adequately been described using a two-compartment open model in dogs (Kukanich and Papich, 2004). Previous studies have contributed to understanding the pharmacokinetics of tramadol and the relationship between its concentrations in blood and its pharmacological effects in dogs. mainly through intravenous, intramuscular, oral and epidural administration (Habibian Dehkordi *et al.*, 2010; McMillan *et al*., 2008; Sousa *et al*., 2008). It has, however, been documented that subcutaneous administration of drugs can achieve the same result as the intramuscular and intravenous routes, with fewer complications, less need for monitoring, and better patient compliance (Sofia *et al*., 2005).

Preliminary data on tramadol's pharmacokinetic behaviour in dogs has confirmed that the extradural (ED) route is equally effective for providing analgesia for surgical techniques (Vettorato *et al.,* 2006). Several studies have described the pharmacokinetics of tramadol following intravenous (McMillan *et al.*, 2008; Kukanich and Papich, 2004), oral (Giorgi *et al.*, 2009; Kukanich and Papich, 2004), intramuscular (Sousa *et al.*, 2008) and epidural routes (Vettorato *et al.*, 2010). Despite the wide clinical applications of tramadol via subcutaneous route in

veterinary clinics at pre- and post-surgery in dogs, its pharmacokinetic behaviour and, in particular, the effect of surgery as well as gender-related variations on the distribution, metabolism and excretion of the drug following subcutaneous administration are not well documented. It was therefore hypothesized that subcutaneous administration of tramadol can provide an equal analgesia to the intravenous route of administration, and that first and second doses administration of tramadol can provide post-surgical analgesia in dogs without pathological effects. Surgery and gender-differences may also influence the pharmacokinetic parameters of tramadol in dogs. Thus, the objectives of this study were to:

1) assess the analgesic efficacy of tramadol given subcutaneously and intravenously for acute pain and inflammation associated with surgery in dogs
2) evaluate renal and hepatic changes after single and second doses of tramadol administration in dogs during surgery
3) determine the pharmacokinetics of tramadol following subcutaneous and intravenous administration in dogs based on the influence of surgery and gender differences.

REFERENCES

Abu-Seida, A.M.A. (2012). Efficacy of diclofenac sodium, either alone or together with cefotaxime sodium, for control of postoperative pain, in dogs undergoing ovariohysterectomy. *Asian Journal of Animal and Veterinary Advances,* 7, 180-186.

Aida, S. and Shimoji, K. (2000). Pre-emptive analgesia: recent findings. *Pain Reviews, 7*(2), 105-117.

Almeida, R. M., Escobar, A. and Maguilnik, S. (2010). Comparison of analgesia provided by lidocaine, lidocaine–morphine or lidocaine–tramadol delivered epidurally in dogs following orchiectomy. *Veterinary Anaesthesia and Analgesia, 37*(6), 542-549.

Andrews, H. L. and Workman, W. (1941). Pain Threshold Measurements in the Dog. *Journal of Pharmacology and Experimental Therapeutics, 73*(1), 99-103.

Ardakani, Y. H. and Rouini, M. R. (2007). Pharmacokinetics of tramadol and its three main metabolites in healthy male and female volunteers. *Biopharmaceutics and Drug Disposition, 28*(9), 527-534.

Aretz, J. S. and Geyer, J. (2011). Detection of the CYP1A2 1117C > T polymorphism in 14 dog breeds. *Journal of Veterinary Pharmacology and Therapeutics, 34*(1), 98-100.

Atici, S., Cinel, I., Cinel, L., Doruk, N., Eskandari, G. and Oral, U. (2005). Liver and kidney toxicity in chronic use of opioids: An experimental long term treatment model. *Journal of Biosciences, 30*(2), 245-252.

Bach, S., Noreng, M. F. and Tjéllden, N. U. (1988). Phantom limb pain in amputees during the first 12 months following limb amputation, after preoperative lumbar epidural blockade. *Pain, 33*(3), 297-301.

Bancos, S., Bernard, M. P., Topham, D. J. and Phipps, R. P. (2009). Ibuprofen and other widely used non-steroidal anti-inflammatory drugs inhibit antibody production in human cells. *Cellular Immunology, 258*(1), 18-28.

Barnhart, M. D., Hubbell, J. A. E., Muir, W. W., Sams, R. A. and Bednarski, R. M. (2000). Pharmacokinetics, pharmacodynamics, and analgesic effects of morphine after rectal, intramuscular, and intravenous administration in dogs. *American Journal of Veterinary Research, 61*(1), 24-28.

Beedham, C. (1997). The role of non—P450 enzymes in drug oxidation. *Pharmacy World and Science,* 19(6), 255-263.

Bianchi, M., Broggini, M., Balzarini, P., Baratelli, E., Ferrario, P. and Panerai, A. E. (2003). Effects of tramadol on synovial fluid concentrations of substance P and interleukin-6 in patients with knee osteoarthritis: comparison with paracetamol. *International Immunopharmacology, 3*(13-14), 1901-1908.

Bianchi, M., Maggi, R., Pimpinelli, F., Rubino, T., Parolaro, D., Poli, V., Ciliberto, G., Panerai, A. E. and Sacerdote, P. (1999). Presence of a reduced opioid response in interleukin-6 knock out mice. *European Journal of Neuroscience, 11*(5), 1501-1507.

Bigham, A. S., Habibian, S., Ghasemian, F. and Layeghi, S. (2010). Caudal epidural injection of lidocaine, tramadol, and lidocaine–tramadol for epidural anaesthesia in cattle. *Journal of Veterinary Pharmacology and Therapeutics, 33*(5), 439-443.

Brodin, E., Gazelius, B., Panopoulos, P. and Olgart, L. (1983). Morphine inhibits substance P release from peripheral sensory nerve endings. *Acta Physiologica Scandinavica, 117*(4), 567-570.

Brondani, J. T., Loureiro Luna, S. P., Beier, S. L., Minto, B. W. and Padovani, C. R. (2009). Analgesic efficacy of perioperative use of vedaprofen, tramadol or their combination in cats undergoing ovariohysterectomy. *Journal of Feline Medicine and Surgery, 11*(6), 420-429.

Brown, D. C., Bernier, N., Shofer, F., Steinberg, S. A. and Perkowski, S. Z. (2002). Use of noninvasive dental dolorimetry to evaluate analgesic effects of intravenous and intrathecal administration of morphine in anesthetized dogs. *American Journal of Veterinary Research, 63*(10), 1349-1353. doi: 10.2460/ajvr.2002.63.1349

Budsberg, S. C. (2001). Long-term temporal evaluation of ground reaction forces during development of experimentally induced osteoarthritis in dogs. *American Journal of Veterinary Research, 62*(8), 1207-1211.

Buhagiar, L., Cassar, O. A., Brincat, M. P., Buttigieg, G. G., Inglott, A. S., Adami, M. Z. and Azzopardi, L. M. (2011). Predictors of post-caesarean section pain and analgesic consumption. *Journal of anaesthesiology, clinical pharmacology*, *27*(2), 185-191.

Cagnardi, P., Villa, R., Zonca, A., Gallo, M., Beccaglia, M., Luvoni, G. C., Vettorato, E., Carlis, S., Fonda, D. and Ravasio, G. (2011). Pharmacokinetics, intraoperative effect and postoperative analgesia of tramadol in cats. *Research in Veterinary Science*, *90*(3), 503-509.

Caulkett, N., Read, M., Fowler, D. and Waldner, C. (2003). A comparison of the analgesic effects of butorphanol with those of meloxicam after elective ovariohysterectomy in dogs. *The Canadian veterinary journal. La revue veterinaire canadienne, 44*(7), 565-570.

Chapman, V., Suzuki, R. and Dickenson, A. H. (1998). Electrophysiological characterization of spinal neuronal response properties in anaesthetized rats after ligation of spinal nerves L5-L6. *The Journal of Physiology, 507*(3), 881-894.

Chauret, N., Gauthier, A., Martin, J. and Nicoll-Griffith, D. A. (1997). In Vitro Comparison of Cytochrome P450-Mediated Metabolic Activities in Human, Dog, Cat, and Horse. *Drug Metabolism and Disposition, 25*(10), 1130-1136.

Clifford, J. W. (2004). Pain: Moving from Symptom Control toward Mechanism-Specific Pharmacologic Management. *Annals of Internal Medicine, 140*(6), 441.

Conzemius, M. G., Hill, C. M., Sammarco, J. L. and Perkowski, S. Z. (1997). Correlation between subjective and objective measures used to determine severity of postoperative pain in dogs. *Journal of the American Veterinary Medical Association, 210*(11), 1619-1622.

Craig, A. D. and Dostrovsky, J. O. (1999) Medulla to thalamus. In Textbook of Pain. 4th edn. Eds. Wall PD. Melzak R. Churchill Livingstone, London. Pp. 183-214.

Crighton, I. M., Martin, P. H., Hobbs, G. J., Cobby, T. F., Fletcher, A. J. and Stewart, P. D. (1998). A comparison of the effects of intravenous tramadol, codeine, and morphine on gastric emptying in human volunteers. *Anaesthesia and Analgesia, 87*(2), 445-449.

Crile, G. (1913). The Kinetic Theory of Shock and its Prevention Through Anoci-Association (Shockless Operation). *The Lancet, 182*(4688), 7-16.

Cruickshank, A. M., Fraser, W. D., Burns, H. J., Van Damme, J. and Shenkin, A. (1990). Response of serum interleukin-6 in patients undergoing elective surgery of varying severity. *Clin. Sci., 79*(2), 161-165.

Cunha, F. Q., Poole, S., Lorenzetti, B. B. and Ferreira, S. H. (1992). The pivotal role of tumour necrosis factor alpha in the development of inflammatory hyperalgesia. *British Journal of Pharmacology, 107*(3), 660-664.

Davidson, E. B., David moll, H. and Payton, M. E. (2004). Comparison of Laparoscopic Ovariohysterectomy and Ovariohysterectomy in Dogs. *Veterinary Surgery, 33*(1), 62-69.

Dayer, P., Collart, L. and Desmeules, J. (1994). The pharmacology of tramadol. *Drugs, 47 Suppl 1*, 3-7.

De Jongh, R. F., Vissers, K. C., Meert, T. F., Booij, L. H. D. J., De Deyne, C. S. and Heylen, R. J. (2003). The Role of Interleukin-6 in Nociception and Pain. *Anaesthesia and Analgesia, 96*(4), 1096-1103.

De Riu, P., Petruzzi, V., Palmieri, G., Gentili, C., Melis, F., Caria, M. A., Azzena, G. B., Casu, A. R., Marras, G. and Madeddu, G. (1989). Beta-endorphin in experimental canine spinal ischemia. *Stroke, 20*(2), 253-258.

De Witte, J. L., Schoenmaekers, B., Sessler, D. I. and Deloof, T. (2001). The Analgesic Efficacy of Tramadol is Impaired by Concurrent Administration of Ondansetron. *Anaesthesia and Analgesia, 92*(5), 1319-1321.

Derbyshire, S. W. G., Jones, A. K. P., Gyulai, F., Clark, S., Townsend, D. and Firestone, L. L. (1997). Pain processing during three levels of noxious stimulation produces differential patterns of central activity. *Pain, 73*(3), 431-445.

Dickenson, A. H. (1991). Mechanisms of the analgesic actions of opiates and opioids. *British Medical Bulletin, 47*(3), 690-702.

Dickenson, A. H. (1995). Spinal cord pharmacology of pain. *British Journal of Anaesthesia, 75*(2), 193-200.

Dickenson, A. H. and Sullivan, A. F. (1987). Peripheral origins and central modulation of subcutaneous formalin-induced activity of rat dorsal horn neurones. *Neuroscience Letters, 83*(1-2), 207-211.

Djurendic-Brenesel, M., Mimica-Dukic, N., Pilija, V. and Tasic, M. (2010). Gender-related differences in the pharmacokinetics of opiates. *Forensic Science International, 194*(1–3), 28-33.

Dray, A. (1995). Inflammatory mediators of pain. *British Journal of Anaesthesia, 75*(2), 125-131.

Dreyer, W. J., Phillips, S. C., Lindsey, M. L., Jackson, P., Bowles, N. E., Michael, L. H. and Entman, M. L. (2000). Interleukin 6 induction in the canine myocardium after cardiopulmonary bypass. *J Thorac Cardiovasc Surg, 120*(2), 256-263.

Duggan, A. W. and North, R. A. (1983). Electrophysiology of opioids. *Pharmacological Reviews, 35*(4), 219-281.

Edwards, L., Ring, C., France, C. R., al'Absi, M., McIntyre, D., Carroll, D. and Martin, U. (2007). Nociceptive flexion reflex thresholds and pain during rest and computer game play in patients with hypertension and individuals at risk for hypertension. *Biological Psychology, 76*(1-2), 72-82.

Elghazali, M., Barezaik, I. M., Abdel Hadi, A. A., Eltayeb, F. M., Al Masri, J. and Wasfi, I. A. (2008). The pharmacokinetics, metabolism and urinary detection time of tramadol in camels. *The Veterinary Journal, 178*(2), 272-277.

Ferreira, S. H., Lorenzetti, B. B., Bristow, A. F. and Poole, S. (1988). Interleukin-1[beta] as a potent hyperalgesic agent antagonized by a tripeptide analogue. [10.1038/334698a0]. *Nature, 334*(6184), 698-700.

Fields, H. (2004). State-dependent opioid control of pain. [10.1038/nrn1431]. *Nat Rev Neurosci, 5*(7), 565-575.

Firth, A. M. and Haldane, S. L. (1999). Development of a scale to evaluate postoperative pain in dogs. *Journal of the American Veterinary Medical Association, 214*(5), 651-659.

Floren, L. C., Bekersky, I., Benet, L. Z., Mekki, Q., Dressler, D., Lee, J. W., Hebert, M. F. (1997). Tacrolimus oral bioavailability doubles with coadministration of ketoconazole[ast]. *Clin Pharmacol Ther, 62*(1), 41-49.

Foulkes, T. and Wood, J. N. (2008). Pain Genes. *PLoS Genet, 4*(7), e1000086.

Franconi, F., Brunelleschi, S., Steardo, L. and Cuomo, V. (2007). Gender differences in drug responses. *Pharmacological Research, 55*(2), 81-95.

Gan, S. H. and Ismail, R. (2001). Validation of a high-performance liquid chromatography method for tramadol and o-desmethyltramadol in human plasma using solid-phase extraction. *Journal of Chromatography B: Biomedical Sciences and Applications, 759*(2), 325-335.

Gan, S. H., Ismail, R., Wan Adnan, W. A. and Wan, Z. (2002). Method development and validation of a high-performance liquid chromatographic method for tramadol in human plasma using liquid-liquid extraction. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 772*(1), 123-129.

Garrido, M. J., Valle, M., Campanero, M. A., Calvo, R. and Trocóniz, I. F. (2000). Modeling of the In Vivo Antinociceptive Interaction between an Opioid Agonist, (+)-O-Desmethyltramadol, and a Monoamine Reuptake Inhibitor, (−)-O-Desmethyltramadol, in Rats. *Journal of Pharmacology and Experimental Therapeutics, 295*(1), 352-359.

Garry, E. M., Jones, E. and Fleetwood-Walker, S. M. (2004). Nociception in vertebrates: key receptors participating in spinal mechanisms of chronic pain in animals. *Brain Research Reviews, 46*(2), 216-224.

Geiss, A., Varadi, E., Steinbach, K., Bauer, H. W. and Anton, F. (1997). Psychoneuroimmunological correlates of persisting sciatic pain in patients who underwent discectomy. *Neuroscience Letters, 237*(2-3), 65-68.

Gibaldi, M. and Perrier, D. (1982). Noncompartmental analysis based on statistical moment theory, p. 409–417. *In* J. Swarbrick (ed.), Pharmacokinetics, 2nd ed. Marcel Dekker, Inc., New York.

Gibson, T. P. (1996). Pharmacokinetics, efficacy, and safety of analgesia with a focus on tramadol HCl. *The American journal of medicine, 101, Supplement 1*(0), S47-S53.

Giorgi, M. (2012). Tramadol Vs Tapentadol: Anew Horizon in Pain Treatment?. *American Journal of Animal and Veterinary Sciences,* 7(1), 7-11.

Giorgi, M., Saccomanni, G., Lebkowska-Wieruszewska, B. and Kowalski, C. (2009). Pharmacokinetic evaluation of tramadol and its major metabolites after single oral sustained tablet administration in the dog: a pilot study. *The Veterinary Journal, 180*(2), 253-255.

Gómez-Lechón, M. J., Ponsoda, X., Jover, R., Fabra, R., Trullenque, R. and Castell, J. V. (1987). Hepatotoxicity of the opioids morphine, heroin, meperidine, and

methadone to cultured human hepatocytes. *Molecular toxicology, 1*(4), 453-463.

González-Darder, J. M., Barberá, J. and Abellán, M. J. (1986). Effects of prior anaesthesia on autotomy following sciatic transection in rats. *pain*, 24(1), 87-91..

Grond, S. and Sablotzki, A. (2004). Clinical Pharmacology of Tramadol. *Clinical Pharmacokinetics, 43*(13), 879-923.

Guedes, A. G. P., Natalini, C. C., Robinson, E. P., Alves, S. D. L. and Oliveira, S. T. (2005). Epidural administration of tramadol as an analgesic technique in dogs submitted to stifle surgery. *The International Journal of Applied Research in Veterinary Medicine, 3* (4), 352-359.

Habibian Dehkordi, S., Bigham Sadegh, A., Abaspour, E., Beigi Brojeni, N., Aali, E. and Sadeghi, E. (2010). Intravenous administration of tramadol hydrochloride in sheep: a haematological and biochemical study. *Comparative Clinical Pathology*, 1-5.

Hardie, E. M., Hansen, B. D., Carroll, G. (1997). Behavioural after ovariohysterectomy in the dog: What's normal? *Applied Animal Behavioural Science*. 51, 111-128.

Hamlin, R. L., Bednarski, L. S., Schuler, C. J., Weldy, P. L. and Cohen, R. B. (1988). Method of objective assessment of analgesia in the dog. *Journal of Veterinary Pharmacology and Therapeutics, 11*(2), 215-220.

Hancock, R. B., Lanz, O. I., Waldron, D. R., Duncan, R. B., Broadstone, R. V. and Hendrix, P. K. (2005). Comparison of Postoperative Pain After Ovariohysterectomy by Harmonic Scalpel-Assisted Laparoscopy Compared with Median Celiotomy and Ligation in Dogs. *Veterinary Surgery, 34*(3), 273-282.

Hansen, B. D. (2003). Assessment of pain in dogs: Veterinary clinical studies. *Journal of Institute for Laboratory Animal Research*, 44(3), 197-205.

Hartwig, A. C. (1991). Peripheral Beta Endorphin and Pain Modulation. *Anaesthesia Progress, 38*(3), 75–78.

Haussler, K. K., Hill, A. E., Frisbie, D. D. and McIlwraith, C. W. (2007). Determination and use of mechanical nociceptive thresholds of the thoracic limb to assess pain associated with induced osteoarthritis of the middle carpal joint in horses. *American Journal of Veterinary Research, 68*(11), 1167-1176.

Hayes, A. G., Skingle, M. and Tyers, M. B. (1986). Alpha-adrenoceptor-mediated antinociception and sedation in the rat and dog. *Neuropharmacology, 25*(4), 391-396.

Hazewinkel, H. A. W., van den Brom, W. E., Theijse, L. F. H., Pollmeier, M. and Hanson, P. D. (2003). Reduced dosage of ketoprofen for the short-term and long-term treatment of joint pain in dogs. *Veterinary Record, 152*(1), 11-14.

Hebert, M. F., Roberts, J. P., Prueksaritanont, T. and Benet, L. Z. (1992). Bioavailability of cyclosporine with concomitant rifampin administration is markedly less than predicted by hepatic enzyme induction. *Clin. Pharm. Ther., 52*(5), 453-457.

Hellyer, P., Rodan, I., Brunt, J., Downing, R., Hagedorn, J. E. and Robertson, S. A. (2007). AAHA/AAFP pain management guidelines for dogs and cats. *Journal of Feline Medicine and Surgery, 9*(6), 466-480.

Holton, L. L., Scott, E. M., Nolan, A. M., Reid, J., Welsh, E., Flaherty, D. (1998). Comparison of three methods used for assessment of pain in dogs. *Journal of American Veterinary Medical Association*. 212(1):61-6.

Holzheimer, R. G. and Steinmetz, W. (2000). Local and systemic concentrations of pro- and anti-inflammatory cytokines in human wounds. *European journal of medical research, 5*(8), 347-355.

Hui-chen, L., Yang, Y., Na, W., Ming, D., Jian-fang, L. and Hong-yuan, X. (2004). Pharmacokinetics of the enantiomers of trans-tramadol and its active metabolite, trans-O-demethyltramadol, in healthy male and female chinese volunteers. *Chirality, 16*(2), 112-118.

Ishak, K., Baptista, A., Bianchi, L., Callea, F., DeGroote, J., Gudat, F., Denk, H. *et al*. (1995). Histological grading and staging of chronic hepatitis. *Journal of Hepatology*, 22 (6), 696-699.

Jensen, M. P., Karoly, P. and Braver, S. (1986). The measurement of clinical pain intensity: A comparison of six methods. *Pain*. 27, 117-126.

Kalso, E., Tramèr, M. R., Carroll, D., McQuay, H. J. and Moore, R. A. (1997). Pain relief from intra-articular morphine after knee surgery: a qualitative systematic review. *Pain, 71*(2), 127-134.

Kalthum Hashim. Ph.D. Thesis. The Clinical Pharmacology of Pethidine Hydrochloride in Dogs. University of Bristol, 1988.

Kang, J. D., Georgescu, H. I., McIntyre-Larkin, L., Stefanovic-Racic, M., Donaldson, W. F. I. and Evans, C. H. (1996). Herniated Lumbar Intervertebral Discs Spontaneously Produce Matrix Metalloproteinases, Nitric Oxide, Interleukin-6, and Prostaglandin E2. *Spine, 21*(3), 271-277.

Karmen, A., Wrohlewski, F. and Ladue, J. (1955). Transaminase activity in human blood. *Journal of Clinical Investigation, 34*, 126-131.

Katz, J., Kavanagh, B. P., Sandler, A. N., Nierenberg, H., Boylan, J. F., Friedlander, M. and Shaw, B. F. (1992). Preemptive analgesia. Clinical evidence of neuroplasticity contributing to postoperative pain. *Anesthesiology, 77*(3), 439-446.

Kawakami, M., Matsumoto, T., Kuribayashi, K. and Tamaki, T. (1999). mRNA expression of interleukins, phospholipase A2, and nitric oxide synthase in the nerve root and dorsal root ganglion induced by autologous nucleus pulposus in the rat. *Journal of Orthopaedic Research, 17*(6), 941-946.

Kayser, V., Besson, J. M. and Guilbaud, G. (1992). Evidence for a noradrenergic component in the antinociceptive effect of the analgesic agent tramadol in an animal model of clinical pain, the arthritic rat. *European Journal of Pharmacology, 224*(1), 83-88.

Keita, A., Pagot, E., Prunier, A. and Guidarini, C. (2010). Pre-emptive meloxicam for postoperative analgesia in piglets undergoing surgical castration. *Veterinary Anaesthesia and Analgesia, 37*(4), 367-374.

Kidd, B. L. and Urban, L. A. (2001). Mechanisms of inflammatory pain. *British Journal of Anaesthesia, 87*(1), 3-11.

Kissin, I. (2000). Preemptive Analgesia. *Anesthesiology, 93*(4), 1138-1143.

Kongara, K., Chambers, J. P. and Johnson, C. B. (2010). Electroencephalographic responses of tramadol, parecoxib and morphine to acute noxious electrical stimulation in anaesthetised dogs. *Research in Veterinary Science, 88*(1), 127-133.

Kongara, K., Chambers, P. and Johnson, C. B. (2009). Glomerular filtration rate after tramadol, parecoxib and pindolol following anaesthesia and analgesia in comparison with morphine in dogs. *Veterinary Anaesthesia and Analgesia, 36*(1), 86-94.

Kristiansson, M., Saraste, L., Soop, M., Sundqvist, K. G. and Thörne, A. (1999). Diminished interleukin-6 and C-reactive protein responses to laparoscopic versus open cholecystectomy. *Acta Anaesthesiologica Scandinavica, 43*(2), 146-152.

Kubota, R., Komiyama, T., Miwa, Y., Ide, T., Toyoda, H., Asanuma, F. and Yamada, Y. (2008). Pharmacokinetics and postoperative analgesia of epidural tramadol: A prospective, pilot study. *Current Therapeutic Research, 69*(1), 49-55.

KuKanich, B. and Papich, M. G. (2004). Pharmacokinetics of tramadol and the metabolite O-desmethyltramadol in dogs. *Journal of Veterinary Pharmacology and Therapeutics, 27*(4), 239-246.

KuKanich, B., Hogan, B. K., Krugner-Higby, L. A. and Smith, L. J. (2008). Pharmacokinetics of hydromorphone hydrochloride in healthy dogs. *Veterinary Anaesthesia and Analgesia, 35*(3), 256-264.

Lamba, V., Panetta, J. C., Strom, S. and Schuetz, E. G. (2010). Genetic Predictors of Interindividual Variability in Hepatic CYP3A4 Expression. *Journal of Pharmacology and Experimental Therapeutics, 332*(3), 1088-1099.

Lamont, L. A., Tranquilli, W. J. and Grimm, K. A. (2000). Physiology of pain. *Veterinary Clinics of North America Small Animal Practice, 30*(4), 703-728.

Lascelles, B. D. X. (1999). Preoperative analgesia—opioids and NSAIDs. W*altham Focus®, 9*(4), 2-9.

Lascelles, B. D. X., Cripps, P. J., Jones, A. and Waterman, A. E. (1997). Post-operative central hypersensitivity and pain: the pre-emptive value of pethidine for ovariohysterectomy. *Pain, 73*(3), 461-471.

Lascelles, B. D. X., Cripps, P. J., Jones, A. and Waterman-Pearson, A. E. (1998). Efficacy and Kinetics of Carprofen, Administered Preoperatively or Postoperatively, for the Prevention of Pain in Dogs Undergoing Ovariohysterectomy. *Veterinary Surgery, 27*(6), 568-582.

Lascelles, B. D. X., Waterman, A. E., Cripps, P. J., Livingston, A. and Henderson, G. (1995). Central sensitization as a result of surgical pain: investigation of the pre-emptive value of pethidine for ovariohysterectomy in the rat. *Pain, 62*(2), 201-212.

Lascelles, B., Butterworth, S. and Waterman, A. (1994). Postoperative analgesic and sedative effects of carprofen and pethidine in dogs. *Veterinary Record, 134*(8), 187-191.

Lauren, A.T. (2006). Cytochrome P450 and its role in veterinary drug interactions. *Veterinary Clinic: Small Animal Practice*. 36, 975-985.

Le Bars, D., Gozariu, M. and Cadden, S. W. (2001). Animal Models of Nociception. *Pharmacological Reviews, 53*(4), 597-652. `

Lemke, K. A., Runyon, C. L. and Horney, B. S. (2002). Effects of preoperative administration of ketoprofen on anesthetic requirements and signs of postoperative pain in dogs undergoing elective ovariohysterectomy. *Journal of the American Veterinary Medical Association, 221*(9), 1268-1275.

Lewis, K. and Han, N. (1997). Tramadol: a new centrally acting analgesic. *American Journal of Health-System Pharmacy, 54*(6), 643-652.

Lintz, W., Erlacin, S., Frankus, E. and Uragg, H. (1981). Biotransformation of tramadol in man and animal (author's transl). *Arzneimittel-Forschung, 31*(11), 1932-1943.

Liu, Hui-Chen, JIN, Shu-Min, Wang, and Yong-Li. (2003a). Gender-related differences in pharmacokinetics of enantiomers of trans-tramadol and its active metabolite, trans-O-demethyltramadol, in rats. *24*(12), 1265-1269.

Liu, W., Burton-Wurster, N., Glant, T. T., Tashman, S., Sumner, D. R., Kamath, R. V., Lust, G., Kimura, J. H. and Cs-Szabo, G. (2003b). Spontaneous and experimental osteoarthritis in dog: Similarities and differences in proteoglycan levels. *Journal of Orthopaedic Research, 21*(4), 730-737.

Liu, Y., Zhu, S., Wang, K., Feng, Z. and Chen, Q. (2008). Effect of tramadol on immune response and nociceptive thresholds in a rat model of incisional pain. *Journal of Zhejiang University Science B*, 9(11), 895-902.

Maris, A. F., Franco, J. L., Mitozo, P. A., Paviani, G., Borowski, C., Trevisan, R., Uliano-Silva, M., Farina, M. and Dafre, A. L. (2010). Gender Effects of Acute Malathion or Zinc Exposure on the Antioxidant Response of Rat Hippocampus and Cerebral Cortex. *Basic and Clinical Pharmacology and Toxicology, 107*(6), 965-970.

Mark J, M. (1999). The induction of pain: an integrative review. *Progress in Neurobiology, 57*(1), 1-164.

Markantonis, S. L., Kostopanagiotou, G., Panidis, D., Smirniotis, V. and Voros, D. (2004). Effects of blood loss and fluid volume replacement on serum and tissue gentamicin concentrations during colorectal surgery. *Clinical Therapeutics, 26*(2), 271-281.

Markenson, J. A. (1996). Mechanisms of chronic pain. *The American journal of medicine, 101*, S6-S18.

Marshall, E. K., Jr., . (1913). A new method for the determination of urea in blood. *Journal of Biological Chemistry*, 487.

Martin, W. R., Eades, C. G., Thompson, J. A., Huppler, R. E. and Gilbert, P. E. (1976). The effects of morphine- and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. *Journal of Pharmacology and Experimental Therapeutics, 197*(3), 517-532.

Mastrocinque, S. and Fantoni, D. T. (2003). A comparison of preoperative tramadol and morphine for the control of early postoperative pain in canine ovariohysterectomy. *Veterinary Anaesthesia and Analgesia, 30*(4), 220-228.

Mathews, K. A. (2000). Pain assessment and general approach to management. *Veterinary Clinics of North America: Small Animal Practice*, 30, 729-756.

Matthiesen, T., Wöhrmann, T., Coogan, T. P. and Uragg, H. (1998). The experimental toxicology of tramadol: an overview. *Toxicology Letters, 95*(1), 63-71.

McCann, M. E., Donald, R., Zhang, D., Brideau, C., Black, W. C., Hanson, P. D. and Hickey, G. J. (2004). In vitro effects and in vivo efficacy of a novel cyclooxygenase-2 inhibitor in dogs with experimentally induced synovitis. *American Journal of Veterinary Research, 65*(4), 503-512.

McCarty, D. J., Phelps, P. and Pyenson, J. (1966). Crystal-induced inflammation in canine joints. I. An experimental model with quantification of the host response. *The Journal of experimental medicine, 124*(1), 99-114.

McMillan, C. J., Livingston, A., Clark, C. R., Dowling, P. M., Taylor, S. M., Duke, T. and Terlinden, R. (2008). Pharmacokinetics of intravenous tramadol in dogs. *Canadian Journal of Veterinary Research, 72*(4), 325–331.

Mellor, D. J., Stafford, K. J., Todd, S. E., Lowe, T. E., Gregory, N. G., Bruce, R. A. and Ward, R. N. (2002). A comparison of catecholamine and cortisol responses of young lambs and calves to painful husbandry procedures. *Australian Veterinary Journal, 80*(4), 228-233.

Mercadante, S. and Arcuri, E. (2004). Opioids and renal function. *The Journal of Pain, 5*(1), 2-19.

Miranda, H. F., Noriega, V., Olavarria, L., Zepeda, R. J., Sierralta, F. and Prieto, J. C. (2011). Antinociception and Anti-Inflammation Induced by Simvastatin in Algesiometric Assays in Mice. *Basic and Clinical Pharmacology and Toxicology, 109*(6), 438-442.

Mise, M., Hashizume, T. and Komuro, S. (2008). Characterization of Substrate Specificity of Dog CYP1A2 Using CYP1A2-Deficient and Wild-Type Dog Liver Microsomes. *Drug Metabolism and Disposition, 36*(9), 1903-1908.

Moak, P., Hosgood, G., Rowe, E. and Lemke, K. A. (2010). Evaluation of intra-articular and subcutaneous administration of meloxicam for postoperative analgesia following stifle surgery in dogs. *Veterinary and Comparative Orthopaedics and Traumatology, 24*(1), 32-38.

Moalem, G. and Tracey, D. J. (2006). Immune and inflammatory mechanisms in neuropathic pain. *Brain Research Reviews, 51*(2), 240-264.

Møiniche, S., Kehlet, H. and Dahl, J. B. (2002). A Qualitative and Quantitative Systematic Review of Preemptive Analgesia for Postoperative Pain Relief: The Role of Timing of Analgesia. *Anesthesiology, 96*(3), 725-741.

Moll, X., Fresno, L., García, F., Prandi, D. and Andaluz, A. (2011). Comparison of subcutaneous and transdermal administration of buprenorphine for pre-emptive analgesia in dogs undergoing elective ovariohysterectomy. *The Veterinary Journal,* 187(1), 124–128.

Molony, V. (1997). Comments on Anand and Craig (Letters to the Editor). *Pain,* 70, 293.

Morrow, T. J., Paulson, P. E., Danneman, P. J. and Casey, K. L. (1998). Regional changes in forebrain activation during the early and late phase of formalin nociception: analysis using cerebral blood flow in the rat. *Pain, 75*(2-3), 355-365.

Morton, C. M., Reid, J., Scott, E. M., Holton, L. L. and Nolan, A. M. (2005). Application of a scaling model to establish and validate an interval level pain scale for assessment of acute pain in dogs. *American Journal of Veterinary Research, 66*(12), 2154-2166.

Mosley, C. (2011). Pain and Nociception in Reptiles. *Veterinary Clinics of North America: Exotic Animal Practice,* 14, 45-60.

Murthy, B. V., Pandya, K. S., Booker, P. D., Murray, A., Lintz, W. and Terlinden, R. (2000). Pharmacokinetics of tramadol in children after i.v. or caudal epidural administration. *British Journal of Anaesthesia, 84*(3), 346-349.

Natalini, C. C. and Robinson, E. P. (2000). Evaluation of the analgesic effects of epidurally administered morphine, alfentanil, butorphanol, tramadol, and U50488H in horses. *American Journal of Veterinary Research, 61*(12), 1579-1586.

Negro, S., Martín, A., Azuara, M. L., Sánchez, Y. and Barcia, E. (2005). Stability of Tramadol and Haloperidol for Continuous Subcutaneous Infusion at Home. *Journal of Pain and Symptom Management, 30*(2), 192-199.

Neuner, P., Klosner, G., Schauer, E., Pourmojib, M., Macheiner, W., Grünwald, C., Knobler, R., Schwarz, A., Luger, T. A. and Schwarz, T. (1994). Pentoxifylline in vivo down-regulates the release of IL-1 beta, IL-6, IL-8 and tumour necrosis factor-alpha by human peripheral blood mononuclear cells. *Immunology, 83*(2), 262-267.

Niv, D., Whitwam, J. G. and Loh, L. (1983). Depression of Nociceptive Sympathetic Reflexes by the Intrathecal Administration of Midazolam. *British Journal of Anaesthesia, 55*(6), 541-547.

O'Benar, J. D., Hannon, J. P., Peterson, J. L. and Bossone, C. A. (1987). Beta-endorphin, ACTH, and cortisol response to hemorrhage in conscious pigs. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 252*(5), R953-R958.

Olson, P. N. Johnston, S. D. (1993). New developments in small animal population control. *Journal of American Veterinary Medical Association*, 202(6), 904-909.

Panchenko, L. F., Pirozhkov, S. V., Nadezhdin, A. V., Baronets, V. and Usmanova, N. N. (1999). Lipid peroxidation, peroxyl radical-scavenging system of plasma and liver and heart pathology in adolescence heroin users. *Voprosy Meditsinskoi Khimii, 45*(6), 501-506.

Pieper, K., Schuster, T., Levionnois, O., Matis, U. and Bergadano, A. (2011). Antinociceptive efficacy and plasma concentrations of transdermal buprenorphine in dogs. *The Veterinary Journal, 187*(3), 335-341.

Polianskis, R., Graven-Nielsen, T. and Arendt-Nielsen, L. (2002). Spatial and temporal aspects of deep tissue pain assessed by cuff algometry. *Pain, 100*(1), 19-26.

Poulsen, L., Arendt-Nielsen, L., Brosen, K. and Sindrup, S. H. (1996). The hypoalgesic effect of tramadol in relation to CYP2D6[ast]. *Clin Pharmacol Ther, 60*(6), 636-644.

Pypendop, B. H. and Ilkiw, J. E. (2008). Pharmacokinetics of tramadol, and its metabolite, *O*-desmethyltramadol in cats, *J. Vet. Pharmacol. Therap*. 31, 52-59.

Quandt, J. E., Raffe, M. R., Reeh, E. and Wyatt, R. (1994). Evaluation of a microstrain gauge algesimeter for quantitative measurement of nociception in the dog. *Journal of Veterinary Pharmacology and Therapeutics, 17*(5), 399-402.

Raffa, R. B. (2001). Pharmacology of oral combination analgesics: rational therapy for pain. *Journal of Clinical Pharmacy and Therapeutics, 26*(4), 257-264.

Rang, H. P. (2003). Drug elimination and pharmacokinetics. In: Pharmacology. 5th edn. Eds. Rang, H. P., Dale, M. M., Ritter, J. M. and Moore, P. Churchill Livingstone. UK. Pp. 106-119.

Rang, H. P., Dale, M. M., Ritter, J. M., Gardner, P. (1995). Pharmacology. New York: Churchill Livingstone Inc. 66-97; 269-300.

Rasmussen, N. A. and Farr, L. A. (2009). Beta-endorphin response to an acute pain stimulus. *Journal of Neuroscience Methods, 177*(2), 285-288.

Reid, J., Nolan, A. M., Hughes, J. M. L., Lascelles, D., Pawson, P. and Scott, E. M. (2007). Development of the short-form Glasgow Composite Measure Pain Scale (CMPS-SF) and derivation of an analgesic intervention score. *Animal Welfare*, 16(8), 97-104.

Renberg, W. C., Johnston, S. A., Carrig, C. B., Budsberg, S. C., Ye, K. and Veit, H. P. (2000). Evaluation of a method for experimental induction of osteoarthritis of the hip joints in dogs. *American Journal of Veterinary Research, 61*(5), 484-491.

Rexed, B. (1952). The cytoarchitectonic organization of the spinal cord in the cat. *The Journal of Comparative Neurology, 96*(3), 415-495.

Rialland, P., Authier, S., Gauvin, D., Veilleux-Lemieux, D., Franck, D., Fournier, S. and Troncy, E. (2010). Physiologic responses and pain-related behaviors in beagle dogs undergoing trochleoplasty surgery. *5*(3), 156.

Rice, A. S. C., Cimino-Brown, D., Eisenach, J. C., Kontinen, V. K., Lacroix-Fralish, M. L., Machin, I., Mogil, J. S. and Stöhr, T. (2008). Animal models and the prediction of efficacy in clinical trials of analgesic drugs: A critical appraisal and call for uniform reporting standards. *Pain, 139*(2), 243-247.

Riviere, J. E. and Papich, M. G. (2001). Potential and problems of developing transdermal patches for veterinary applications. *Advanced Drug Delivery Reviews, 50*(3), 175-203.

Rosenmann, E., Dishon, T., Durst, A. and Boss, J. H. (1972). Kidney and liver damage following anaesthesia with ether and pentobarbitone. *British Journal of Anaesthesia, 44*(5), 465-468.

Roughan, J. V. and Flecknell, P. A. (2003). Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *European Journal of Pain, 7*(5), 397-406.

Rowland, M. and Tozer, T. N. (1995) *Clinical Pharmacokinetics*, 3rd ed, pp 137–155, Williams and Wilkins, Philadelphia.

Sato, M., Tanaka, S., Suzuki, K., Kohama, A. and Fujii, C. (1989). Complications associated with barbiturate therapy. *Resuscitation, 17*(3), 233-241.

Sawyer, D. C., Rech, R. H., Durham, R. A., Adams, T., Richter, M. A. and Striler, E. L. (1991). Dose response to butorphanol administered subcutaneously to increase visceral nociceptive threshold in dogs. *American journal of veterinary research, 52*(11), 1826-1830.

Schindhelm, R. K., Dekker, J. M., Nijpels, G., Bouter, L. M., Stehouwer, C. D. A., Heine, R. J. and Diamant, M. (2007). Alanine aminotransferase predicts coronary heart disease events: A 10-year follow-up of the Hoorn Study. *Atherosclerosis, 191*(2), 391-396.

Scholz, J. and Woolf, C. J. (2002). Can we conquer pain? *Nature Neuroscience,* 5(11), 1062-1067.

Scott, L. J. and Perry, C. M. (2000). Tramadol: A Review of its Use in Perioperative Pain. *Drugs, 60*(1), 139-176.

Seddighi, M. R., Egger, C. M., Rohrbach, B. W., Cox, S. K. and Doherty, T. J. (2009). Effects of tramadol on the minimum alveolar concentration of sevoflurane in dogs. *Veterinary Anaesthesia and Analgesia, 36*(4), 334-340.

Seltzer, Z. e., Beilin, B., Ginzburg, R., Paran, Y. and Shimko, T. (1991). The role of injury discharge in the induction of neuropathic pain behavior in rats. *Pain, 46*(3), 327-336.

Shah, S. S., Sanda, S., Regmi, N. L., Sasaki, K. and Shimoda, M. (2007). Characterization of cytochrome P450-mediated drug metabolism in cats. *Journal of Veterinary Pharmacology and Therapeutics, 30*(5), 422-428.

Shahed, A. R. and Shoskes, D. A. (2001). Correlation of β-endorphin and prostaglandin e2 levels in prostatic fluid of patients with chronic prostatitis with diagnosis and treatment response. *The Journal of urology, 166*(5), 1738-1741.

Shilo, Y., Britzi, M., Eytan, B., Lifschitz, T., Soback, S. and Steinman, A. (2008) Pharmacokinetics of tramadol in horses after intravenous, intramuscular and oral administration. *J. Vet. Pharmacol. Therap*. 31, 60-65.

Short, C. E., Raeihae, J. E., Raeihae, M. P. and Otto, K. (1992). Comparison of neurologic responses to the use of medetomidine as a sole agent or preanesthetic in laboratory beagles. *Acta Veterinaria Scandinavica, 33*(1), 77-88.

Shutt, D. A., Connell, R. and Fell, L. R. (1989). Effects of ovine corticotropin-releasing factor and vasopressin on plasma β-endorphin, cortisol and behavior after minor surgery in sheep. *Life Sciences, 45*(3), 257-262.

Shutt, D., Fell, L., Connell, R., Bell, A., Wallace, C. and Smith, A. (1987). Stress-induced Changes in Plasma Concentrations of Immunoreactive ÃŸ-Endorphin and Cortisol in Response to Routine Surgical Procedures in Lambs *Australian Journal of Biological Sciences, 40*(1), 97-104.

Sivilotti, L. G., Gerber, G., Rawat, B. and Woolf, C. J. (1995). Morphine Selectively Depresses the Slowest, NMDA-independent Component of C-fibre-evoked Synaptic Activity in the Rat Spinal Cord In Vitro. *European Journal of Neuroscience, 7*(1), 12-18.

Slingsby, L. S. and Waterman-Pearson, A. E. (2001). Analgesic effects in dogs of carprofen and pethidine together compared with the effects of either drug alone. *Veterinary Record, 148*(14), 441-444.

Smith, R., Grossman, A., Boyce, M. J., Besser, G. M. and Rees, L. H. (1985). Effect of histamine infusion on circulating methionine-enkephalin and catecholamine concentrations. *Neuroscience Letters, 55*(3), 289-292.

Sofı´a N., Alicia M., Marı´a L. A., Yolanda S. and Emilia B. (2005). Stability of Tramadol and Haloperidol for Continuous Subcutaneous Infusion at Home. *Journal of Pain and Symptom Management.* 30 (2): 192-199.

Sommer, C. and Kress, M. (2004). Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neuroscience Letters, 361*(1-3), 184-187.

Soldin, O. P. and Mattison, D. R. (2009). Sex Differences in Pharmacokinetics and Pharmacodynamics. *Clinical Pharmacokinetics, 48*(3), 143-157.

Sotgiu, M. L. and Biella, G. (2000). Differential effects of MK-801, a N-methyl-d-aspartate non-competitive antagonist, on the dorsal horn neuron hyperactivity and hyperexcitability in neuropathic rats. *Neuroscience Letters, 283*(2), 153-156.

Sousa, A. B. d., Santos, A. C. D. d., Florio, J. C. and Spinosa, H. d. S. (2008). Pharmacokinetics of tramadol administered by intravenous and intramuscular routes to female dogs submitted to ovariohysterectomy. *Brazilian Journal of Veterinary Research and Animal Science, 45*, 239-247.

Souza, M. J., Greenacre, C. B. and Cox, S. K. (2008). Pharmacokinetics of orally administered tramadol in domestic rabbits (Oryctolagus cuniculus). *American Journal of Veterinary Research, 69*(8), 979-982.

Steagall, P. V. M., Taylor, P. M., Brondani, J. T., Luna, S. P. L., Dixon, M. J. and Ferreira, T. H. (2007). Effects of buprenorphine, carprofen and saline on thermal and mechanical nociceptive thresholds in cats. *Veterinary Anaesthesia and Analgesia, 34*(5), 344-350.

Stein, C. and Machelska, H. (2011). Modulation of Peripheral Sensory Neurons by the Immune System: Implications for Pain Therapy. *Pharmacological Reviews, 63*(4), 860-881.

Sue, D., Salazar, T. A., Turley, K. and Guglielmo, B. J. (1989). Effect of surgical blood loss and volume replacement on antibiotic pharmacokinetics. *Ann Thorac Surg, 47*(6), 857-859.

Tanaka, E. (1999). Gender-related differences in pharmacokinetics and their clinical significance. *Journal of Clinical Pharmacy and Therapeutics, 24*(5), 339-346.

Tasaki, T., Nakamura, A., Itoh, S., Ohashi, K., Yamamoto, Y., Masuda, M., Iwata, H., Kazusake, A., Kamataki, T. and Fujita, S. (1998). Expression and characterization of dog CYP2D15 Using baculovirus expression system. *The Journal of Biochemistry, 123*(1), 162-168.

Taylor, P. (2003). Pain Management in Dogs and Cats - More Causes and Locations to Contemplate. *The Veterinary Journal, 165*(3), 186-187.

Theresa, W. F. (Ed.). (2007). *Small animal surgery* (3 ed.): Mosby Elsevier.

Timbrell JA. Principles of the Biochemical Toxicology. London: Taylor and Francis Ltd 1985: 1-5; 51-63.

Torunn, K. F. (2010). Norwegian School of Veterinary Science. "Pain therapy for piglets." *ScienceDaily*, 11 Oct. 2010.

Toutain, P. L., Cester, C. C., Haak, T. and Laroute, V. (2001). A pharmacokinetic/pharmacodynamic approach vs. a dose titration for the determination of a dosage regimen: the case of nimesulide, a Cox-2 selective nonsteroidal anti-inflammatory drug in the dog. *Journal of Veterinary Pharmacology and Therapeutics, 24*(1), 43-55.

Tume, R. K. and Shaw, F. D. (1992). Beta-endorphin and cortisol concentrations in plasma of blood samples collected during exsanguination of cattle. *Meat Science, 31*(2), 211-217.

Tuncer, S., Pirbuadak, L., Balat, O. and Capar, M. (2003). Adding ketoprofen to intravenous partient-controlled analgesia with tramadol after major gynaecological cancer surgery: a double blinded, randomized, placebo-controlled clinical trial. *Eur J Gynaecol Oncol*. 24: 181-184.

Turker, G., Goren, S., Bayram, S., Sahin, S. and Korfali, G. (2005). Comparison of Lumbar Epidural Tramadol and Lumbar Epidural Morphine for Pain Relief After Thoracotomy: A Repeated-Dose Study. *Journal of Cardiothoracic and Vascular Anaesthesia, 19*(4), 468-474.

Tyner, L. C., Woody, J. B., Reid, J. C., Chafetz, E. P., Lederer, H. A., Keefe, T. J. and Jochle, W. (1997). Multicenter Clinical Comparison Of Sedative And Analgesic Effects Of Medetomidine And Xylazine In Dogs. *American Veterinary Medical Association,* 211(11), 5.

Valtolina, C., Robben, J. H., Uilenreef, J., Murrell, J. C., Aspegrén, J., McKusick, B. C. and Hellebrekers, L. J. (2009). Clinical evaluation of the efficacy and safety of a constant rate infusion of dexmedetomidine for postoperative pain management in dogs. *Veterinary Anaesthesia and Analgesia, 36*(4), 369-383.

Valverde, A., Morey, T. E., Hernández, J. and Davies, W. (2003). Validation of several types of noxious stimuli for use in determining the minimum alveolar concentration for inhalation anesthetics in dogs and rabbits. *American Journal of Veterinary Research, 64*(8), 957-962.

Vettorato, E., Zonca, A., Isola, M., Villa, R., Gallo, M., Ravasio, G., Beccaglia, M., Montesissa, C. and Cagnardi, P. (2010). Pharmacokinetics and efficacy of intravenous and extradural tramadol in dogs. *The Veterinary Journal, 183*(3), 310-315.

Viñuela-Fernández, I., Jones, E., Welsh, E. M. and Fleetwood-Walker, S. M. (2007). Pain mechanisms and their implication for the management of pain in farm and companion animals. *The Veterinary Journal, 174*(2), 227-239.

Wada, R. D., Harashima, H., Ebling, W. F., Osaki, E. W. and Stanski, D. R. (1996). Effects of Thiopental on Regional Blood Flows in the Rat. *Anesthesiology, 84*(3), 596-604.

Wagner, A. E. (Ed.). (2002). *Opioids*: Mosby, St. Louis, MO

Waldhoer, M., Bartlett, S. E. and Whistler, J. L. (2004). Opioid receptors. *Annual Review of Biochemistry, 73*(1), 953-990.

Wang, C., Knowles, M. G., Chakrabarti, M. K. and Whitwam, J. G. (1994). Clonidine has comparable effects on spontaneous sympathetic activity and afferent A delta and C-fiber-mediated somatosympathetic reflexes in dogs. *Anesthesiology, 81*(3), 710-717.

Wang, G., Weng, Y., Ishiguro, Y., Sakamoto, H. and Morita, S. (2005). The effect of tramadol on serum cytokine response in patients undergoing pulmonary lobectomy. *Journal of Clinical Anaesthesia, 17*(6), 444-450.

Waterman, A. and Kalthum, W. (1989a). Pharmacokinetics of intramuscularly administered pethidine in dogs and the influence of anaesthesia and surgery. *Veterinary Record, 124*(12), 293-296.

Waterman, A. and Kalthum, W. (1989b). The absorption and distribution of subcutaneously administered pethidine in the dog. *Journal of Association of Veterinary Anaesthesia,*16, 51-52.

Welsh, E. M., Nolan, A. M. and Reid, J. (1997). Beneficial effects of administering carprofen before surgery in dogs. *Veterinary Record, 141*(10), 251-253. doi: 10.1136/vr.141.10.251

Wilder-Smith, C. H., Hill, L., Wilkins, J. and Denny, L. (1999). Effects of Morphine and Tramadol on Somatic and Visceral Sensory Function and

Gastrointestinal Motility after Abdominal Surgery. *Anesthesiology, 91*(3), 639.

Willis, W. D. (2005) Physiology and Anatomy of the spinal cord pain system. In: The Paths of Pain 1975-2005. Eds. Merskey H, Loeser JD, Dubner R. IASP Press. Seattle. Pp. 85-100.

Woolf, C. J. and Bromley, L. (1999) Pre-emptive Analgesia by Opioids. In: Opioids in Pain Control; Basic and Clinical Aspects. Ed. Christoph Stein, Cambrdge University Pres. Pp.212-233.

Woolf, C. J. (1983). Evidence for a central component of post-injury pain hypersensitivity. *Nature, 306*(5944), 686-688.

Woolf, C. J. (1995). Somatic pain--pathogenesis and prevention. *British Journal of Anaesthesia, 75*(2), 169-176.

Woolf, C. J. and Chong, M. S. (1993). Preemptive analgesia--treating postoperative pain by preventing the establishment of central sensitization. *Anaesthesia and analgesia, 77*(2), 362-379.

Woolf, C. J. and Costigan, M. (1999). Transcriptional and posttranslational plasticity and the generation of inflammatory pain. *Proceedings of the National Academy of Sciences, 96*(14), 7723-7730.

Woolf, C. J. and Wall, P. D. (1986). Morphine-sensitive and morphine-insensitive actions of C-fibre input on the rat spinal cord. *Neuroscience Letters, 64*(2), 221-225.

Woolf, C. J., Shortland, P. and Coggeshall, R. E. (1992). Peripheral nerve injury triggers central sprouting of myelinated afferents. [10.1038/355075a0]. *Nature, 355*(6355), 75-78.

Wootton, R., Crosst, G., Wood, S. and Westt, C. D. (1988). An analgesiometry system for use in rabbits with some preliminary data on the effects of buprenorphine and lofentanil. *Lab Anim, 22*(3), 217-222.

Wordliczek, J., Banach, M., Garlicki, J., Jakowicka-Wordliczek, J. and Dobrogowski, J. (2002). Influence of pre- or intraoperational use of tramadol (preemptive or preventive analgesia) on tramadol requirement in the early postoperative period. *Polish Journal of Pharmacology, 54*(6), 693-697.

Wordliczek, J., Szczepanik, A. M., Banach, M., Turchan, J., Zembala, M., Siedlar, M., Przewlocki, R., Serendnicki, W. and Przewlocka, B. (2000). The effect of pentoxifiline on post-injury hyperalgesia in rats and postoperative pain in patients. *Life Sciences, 66*(12), 1155-1164.

Wróblewski, F. and LaDue, J. S. (1956). Serum Glutamic Pyruvic Transaminase in Cardiac and Hepatic Disease. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.), 91*(4), 569-571.

Wu, W. N., McKown, L. A., Gauthier, A. D., Jones, W. J. and Raffa, R. B. (2001) Metabolism of the analgesic drug, tramadol hydrochloride, in rat and dog. Xenobiotica, 31, 423–441.

Wunsch, L. A., Schmidt, B. K., Krugner-Higby, L. A. and Smith, L. J. (2010). A comparison of the effects of hydromorphone HCl and a novel extended release hydromorphone on arterial blood gas values in conscious healthy dogs. *Research in Veterinary Science*, 88(1), 154-158.

Yamaoka, K., Nakagawa, T. and Uno, T. (1977). Application of Akaike's Information Criterion (AIC) in the Evaluation of Linear Pharmacokinetic Equations. *Journal of Pharmacokinetics and Biopharmaceutics*, 6(2), 165-175.

Yang, X., Zhang, B., Molony, C., Chudin, E., Hao, K., Zhu, J., Gaedigk, A., Suver, C., Zhong, H., Leeder, J.S., Guengerich, F.P., Strom, S.C., Schuetz, E., Rushmore, T.H., Ulrich, R.G., Slatter, J.G., Schadt, E.E., Kasarskis, A. and Lum, P. Y. (2010). Systematic genetic and genomic analysis of cytochrome P450 enzyme activities in human liver. *Genome Res*. 20, 1020–1036.

Zhang, Y. T., Zheng, Q. S., Pan, J. and Zheng, R. L. (2004). Oxidative Damage of Biomolecules in Mouse Liver Induced by Morphine and Protected by Antioxidants. *Basic and Clinical Pharmacology and Toxicology*, *95*(2), 53-58.