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Acute pain in domestic cats: nociceptive investigation and novel therapeutics

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Résumé

La disponibilité des médicaments analgésiques est limitée en médecine vétérinaire féline. Le but de cette étude était d'investiguer les propriétés anti-nociceptiques d'une nouvelle formule de buprénorphine (Simbadol, 1.8 mg ml^{-1}) et tapentadol chez les chats.

Six chats étaient inclus dans deux études différentes, les deux étant prospectives, randomisées, croisées, et aveuglées. Dans la première étude, Simbadol (1.8 mg mL^{-1}) a été administré par voies sous-cutanée (SC;0.24 mg kg^{-1}), intraveineuse (IV; 0.12 mg kg^{-1}) et buccale (OTM; 0.12 mg kg^{-1}) et les seuils thermiques ont été comparés avec ceux d'un groupe contrôle contenant de la saline (SAL; saline SC). Les concentrations plasmatiques de buprénorphine et norbuprénorphine ont été mesurées jusqu'à 72 heures suivant chaque traitement de buprénorphine. Un modèle pharmacocinétique-pharmacodynamique adapté à 2 substances et 3 voies d'administration a été utilisé. Dans la deuxième étude, les seuils thermiques ont été comparés entre les chats recevant de la buprénorphine (0.02 mg kg^{-1}, IM), un placébo (50 mg de dextrose oral) et deux doses de tapentadol oralement (dose réduite: 25 mg; dose élevée: 50 mg)

L'administration sous-cutanée de Simbadol a provoqué une anti-nociception thermique de longue durée (≥ 24 heures). Ces effets étaient prolongés comparativement aux traitements intraveineux (8 heures) et buccal (12 heures). Le modèle conjoint de pharmacocinétique/pharmacodynamique a démontré des concentrations plasmatiques prolongée pour la voie sous-cutanée. Les deux doses de tapentadol ont augmenté l'antinociception thermique chez les chats. La dose élevée de tapentadol a produit une durée d'antinociception similaire à celle de la buprénorphine (2 heures) et deux fois plus longue que la dose réduite. La palatabilité de la médication représente une limite significative de la voie d'administration.

Simbadol et tapentadol produisent une antinociception thermique comparée à la saline. Des investigations cliniques supplémentaires seront nécessaires.

Mots clés :

Félin, douleur, analgésie, antinociception thermique, opioïde, buprenorphine, tapentadol.

Abstract

Analgesic drug availability is limited in feline practice. The aim of these studies was to investigate the antinociceptive properties of a novel formulation of buprenorphine (Simbadol, 1.8 mg ml^{-1}) and tapentadol in cats.

In two separate studies, six healthy cats (each) were included in a prospective, randomised, blinded, crossover study. In study I, Simbadol (1.8 mg mL^{-1}) was administered by various routes: subcutaneous (SC; 0.24 mg kg^{-1}), intravenous (IV; 0.12 mg kg^{-1}) or buccal (OTM; 0.12 mg kg^{-1}) route of administration and thermal thresholds (TT) were compared with a saline group (SAL; saline SC). Plasma buprenorphine and norbuprenorphine concentrations were measured up to 72 hours following each buprenorphine treatment. A bespoke pharmacokinetic-pharmacodynamic model fitted data from two analytes/three routes of administration. In study II, thermal thresholds were compared among cats receiving buprenorphine (0.02 mg kg^{-1}, IM), placebo (50 mg oral dextrose) and two doses of oral tapentadol (low-dose 25 mg; high-dose 50 mg).

Subcutaneous administration of Simbadol provided long-lasting thermal antinociception (≥ 24 hours). These effects are prolonged compared with the IV (8 hours) and OTM (12 hours) treatments. Joint pharmacokinetic-pharmacodynamic modelling demonstrated prolonged plasma concentrations for the SC route. Both doses of tapentadol increased thermal antinociception in cats. The high-dose of tapentadol produced similar duration of antinociception as intramuscular buprenorphine (2 hours) and twice as long as the low-dose. Palatability presented a significant limitation to the drug's administration.

Simbadol and tapentadol produced thermal antinociception when compared with saline. Additional investigation is necessary to determine if this translates to the clinical setting.

Keywords:

Feline, pain, analgesia, thermal antinociception, opioid, buprenorphine, tapentadol.

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List of Abbreviations

AAFP:	American Association of Feline Practitioners
ANOVA:	Analysis of variance
ARRIVE:	Animal research: Reporting of *in vivo* experiments
BL:	Baseline values
BUP	Buprenorphine 0.02 mg kg^{-1} IM
CMPS-F:	Feline composite measure pain scale with grimace scale
COX:	Cyclooxygenase
FDA:	Food and drug administration
HighTAP	High-dose tapentadol (50mg PO)
HPLC-MS	High performance liquid chromatography-Mass spectrometry
IM:	Intramuscular
ISFM:	International Society of Feline Medicine
IV:	Intravenous
LowTAP	Low-dose tapentadol (25mg PO)
MAC:	Minimum alveolar concentration
NMDA:	*N*-methyl-*D*-aspartate
NSAIDS:	Non-steroidal anti-inflammatory drugs
OTM:	Buccal or Oral Transmucosal
PBO:	Placebo
PD:	Pharmacodynamics
PK:	Pharmacokinetics

PK-PD:	Pharmacokinetics-pharmacodynamics
PO:	Per-os
QST:	Quantitative sensory testing
rCMPS-F:	Feline composite measure pain scale
SAL:	Saline treatment
SC:	Subcutaneous
SD:	Standard Deviation
$t_{1/2}$:	Half-life
TT :	Thermal Threshold
UGTs:	Uridine 5’-diphosphoglucuronosyl transferases
UNESP-MCPS:	UNESP-Botucatu Multidimensional composite pain scale
VAS:	Visual analogue scale

Dedication

To my father,

I could not have achieved all that I have personally, academically, and professionally without your love, wisdom, and support.

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To my supervisor, Dr. Paulo Steagall, for providing me with your support and guidance throughout this program. I am truly amazed at the opportunities and experiences you provided me over the course of this masters. Multiple publications, travelled the world for conferences, and learned about the scientific method along the way. Thank you so much.

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Introduction

The intent of this literature review is to put into perspective the current knowledge regarding acute pain in cats. The beginning is intended to explain practical limitations on veterinary assessments of pain in cats, idiosyncrasies of the species and attitudes towards pain management. Next the review will explore methods of pain quantification and define and explore nociceptive research. The final topic for revision is management of feline acute pain. Comparisons are often made to the dog as a method of providing context to differences observed. Ultimately the review should provide the reader an understanding of nociceptive studies in feline medicine providing the framework for the investigation of the novel treatments in this thesis. The intent of these studies is to improve the understanding and management of acute pain in cats.

1. Acute pain in cats

Cats are the most popular household pet in Canada. There were 7 million cats living in homes across the country as reported by a survey in 2014 (1). Cats are less likely to have regular veterinary visits when compared with dogs. The same owners would pay more for life-saving procedures for dogs versus cats. Socioeconomic factors affected these perceptions, but better client communication was highlighted as essential to improving this disparity (2). When considering acute (adaptive) pain, perioperative or peri-procedural pain is more frequently recognised and treated compared to other manifestations of acute pain. Unfortunately, fewer veterinary visits result in reduced preventive medicine for cats increasing the likelihood of diseases which may produce pain (eg. dental disease, obesity-related osteoarthritis, urinary obstruction, etc.). A better understanding of nociception and pain states, as well as treatment modalities, is vital to this deficit.

1.1 Problems in recognising acute pain

Many characteristics exist with the cat that makes the recognition and management of pain problematic. The behavioural changes associated with veterinary visits has been considered a reason for the decreased frequency of veterinary examinations for the species. Stress and anxiety associated with transport, other animals in waiting areas, as well as restraint and handling during examinations and procedures are often manifested as avoidance or aggressive behaviours in the species. Fear is the most common cause of aggression in cats in the veterinary clinic setting, followed by pain (3). This overlap of behaviours with different causes makes clinical evaluations more complicated and difficult. Challenging behavioural modification techniques exist. The American Association of Feline Practitioners (AAFP) and International Society of Feline Medicine (ISFM) recommend the adoption of friendly handling techniques to minimize the impact of these behaviours (4). These recommendations extend beyond handling techniques and also include recommendations for clients at home before a veterinary visit, changes to clinic environment, and techniques for managing the return of patients to the home following extended hospitalisation. The impact of stress on cats adversely affects sickness-associated behaviours (e.g. vomiting, diarrhoea, decreased activity, enhanced pain-like behaviour, etc.) in both healthy and sick cats as well as their physiologic and haematologic variables (5, 6). These parameters are considered in unison when evaluating the health status of veterinary patients, thus differentiating stress and pain can be difficult.

Idiosyncrasies in drug metabolism have negatively impacted the availability, licensure and use of many analgesics. Classically, the use of non-steroidal anti-inflammatory drugs (NSAIDS) has been cautious due to the reduced capacity for glucuronidation (7-9). Limitations of glucuronidation also affect opioid analgesia. Particularly morphine, where production of the active metabolite morphine-6-glucuronide is limited (10). The behavioural impacts of the agonists of μ-opioid receptors have also

been exaggerated due to initial investigations using doses above those used clinically (at present), producing erratic responses to stimuli and dysphoria (11). These considerations, as well as reduced feline pharmaceutical market demand (compared with dogs) has led to fewer approved analgesics, limiting available therapeutics for cats. Additionally, when a product is licensed for use in cats, it is often only available for single-use or short-term dosing (e.g. meloxicam in North America) requiring "off-label" dosing for long-term usage. For dogs, this "off-label" usage of many drugs is more frequent. This difference is multifactorial due in part to the perceived increased risk of adverse effects in cats, and a greater body of clinical studies on pain management in dogs. This wealth of studies provides better support for unlicensed dosing regimens and therapeutics in dogs (12, 13).

1.2. Attitudes and prevalence of analgesic administration in cats

Since 2000, the perceptions and attitudes pertaining to acute pain management have evolved considerably. In the first ten years of the millennium, the majority of surveys highlighted inadequacies in training and education. There were significant differences in attitudes to analgesia which varied geographically (or perhaps more specifically, culturally). Canadian veterinarians (n = 326) were surveyed in 2001 regarding their perioperative used of analgesics in dogs and cats (14). With respect to ovariohysterectomy, it was shown that more recent graduates, veterinarians with increased personal perception of pain and the presence of animal health technicians increased the likelihood of pain scoring and analgesic administration. Concern regarding the negative aspects (adverse effects and cost) of opioid administration were cited as a reason for avoidance of analgesic administration. Interestingly the same individuals tended to also avoid NSAIDs in the same cases. Another survey was conducted in 2001 of New Zealand Veterinarians (n = 320) (15). Less than half of these practitioners felt their knowledge was adequate for pain management. This study showed that despite procedures being deemed painful, routine analgesics were not always given. Across all common surgical

procedures, dogs received more analgesics than cats (eg. castration, where 65% of dogs and 50% of cats had routine analgesia). Additionally, the negative impact of adverse effects was more frequently considered in the selection of analgesics for cats than dogs. A Finnish study published in 2003 again showed that younger veterinarians (who tended to be women), as well as those who work in larger practices, used more analgesics than older veterinarians and those in smaller clinics (16). Veterinarians in this survey (n = 434) agreed that post-operative pain management was beneficial, a third of respondents considered that a certain degree of post-operative pain was useful for case management. In general, pain was more frequently treated in dogs than cats for ovariohysterectomy and castration. Interestingly, canine castration was deemed as painful as feline ovariohysterectomy (numerical rating scale, scored 6.10 and 6.14, respectively, out of 10), however, castrated dogs were more likely to receive analgesics. When comparing castration alone between the species, results showed less than 10% of respondents would never give analgesics to dogs but more than 60% would not give analgesics to cats. The study concluded that a disparity existed in the knowledge base of these practitioners, and improved education was warranted. In a survey from 2007 of Brazilian veterinarians (n = 1298), it was found that women and younger veterinarians have increased pain recognition (17). For celiotomies, orchiectomies and dental procedures, cats were often regarded as having a lower pain score. The same veterinarians felt their knowledge was inadequate, despite having comparable or better pain recognition than previous studies. In these studies from the early 2000s, lack of analgesic administration is largely attributed to inadequate knowledge and education regarding drug pharmacology and adverse effects.

Over the last seven years, the recognition and attitudes towards acute pain management have improved. Limitations now seem to be less associated with just education but now also include an emphasis on the need for more research in the field to broaden the available knowledge. In a 2010

survey of veterinarians (n = 258) from Switzerland, the veterinarians responded that their justification for analgesic use was based principally on drug efficacy (18). Contradictorily, butorphanol and buprenorphine were the most common opioids used in the country, not the agonists of μ-opioid receptors with superior analgesic efficacy (to be discussed later). The authors suggested a weakness in drug pharmacology that could explain this difference. Though extensive comparisons could not be made due to a small number of respondents, the authors also suggested there should be an increase in the use of pain assessment instruments which are becoming increasingly more available. In 2012 a survey was performed of Canadian veterinarians in Ontario (n = 229) (19). This study found that there was an overall improvement in the awareness and provision of analgesics. The respondents felt their knowledge was sufficient. An interesting shift occurred where there was no longer a perception that cost of drugs or side effects were a sufficient justification for a lack of analgesic administration. In 2013, a survey of United Kingdom veterinarians (n = 720) showed 98% prevalence of perioperative NSAIDS administration for ovariohysterectomy and castration (20). Three quarters of respondents also used a multimodal approach (NSAID + opioid). In this study, there was no difference between year of graduation and analgesic administration. Orthopaedic, abdominal and dental pain were regarded as equal between dogs and cats. The only species difference was that neutering and ovariohysterectomy were considered less painful for cats than dogs, and male vets were less likely to administer NSAIDs to cats (94% vs 99% of women). In a survey published in 2014, attitudes towards feline pain were compared between veterinarians (n = 717) from New Zealand, Australia and the United Kingdom (21). In this study, all three countries had improved peri-operative analgesia compared with previous studies. Despite this, an absence of post-operative drug administration as well as follow-up (at home) analgesia was identified. The authors suggested that more research was required for both post-operative drugs and analgesic timing. It seems that awareness of pain as a problem has improved recently, more research and dissemination of these findings to practitioners is needed.

1.3 Instruments for Acute Pain Assessment

Pain is an inherently subjective experience (22). Methods by which this experience can be quantified allow for better identification and even comparison of pain. These tools then can be used both clinically and in the research setting. The development of a subjective instrument for the assessment of pain requires four steps, chronologically; item generation, readability testing, reliability testing, and validity testing (23). Item generation is the first step, where specific assessments or evaluations are considered for evaluating pain. Readability testing should evaluates wheter a tool is practical and simple to understand and use. Reliability testing evaluates the consistency of measures yielded by the items within the instrument, as well as repeated measures by the same observer, different observers, and the same subject over a series of evaluations (24). The final step, validity testing, can be further subdivided into content, construct, and criterion validity. Content and construct validity evaluate the sensitivity of the items in the instrument to categorize pain states. Whereas criterion validity describes the correlation between an external objective measure and the scale itself (25). Ideally this should be a comparison to a gold-standard test, however no objective measure of pain has been classified as such. Cultural and lingual differences provide language specific readability, reliability and validity testing. The goal of clinical pain instruments is to assist veterinarians in determining whether or not the subject requires analgesia. Intervention thresholds are often determined based on a score which best separates painful animals from non-painful, ideally with the fewest misclassifications. There are several scales which have been developed for dogs for both acute and chronic pain across a number of conditions (26-31). Several of these scales have been evaluated and determined to be valid. There are fewer scales developed for cats limiting the available tools for pain identification and research (32-34).

A recent review evaluated instruments available for the subjective assessment of pain in cats including simple descriptive scales, VAS, numerical rating scales, dynamic interactive visual analogue scale and multidimensional composite scales (24). At present, only two instruments have been evaluated for validity. The feline composite measure pain scale (rCMPS-F) was developed as a clinical tool and validated as such across all breeds, ages and types of acute pain in cats. The validation was limited to content and construct, but did not include criterion validity. The scale includes an intervention point when the score is greater than or equal to 4 out of a possible 16 (33). In the development of this scale, the authors indicated the intention to include a grimace scale in a subsequent revision of the instrument. The inclusion of this grimace scale has now been validated (CMPS-F) (34). In this validation the authors also derived and revised the intervention score (greater than or equal to 5 out of 20). This revised version of the rCMPS-F performed better than the original, misclassifying only 17.6% of painful cats compared to 26.7%. The only other validated instrument is the UNESP-Botucatu Multidimensional composite pain scale (UNESP-MCPS) (32). This instrument was developed initially in Brazilian Portuguese for the evaluation of post-operative pain following ovariohysterectomy (35). This instrument included similar evaluation of pain expression and psychomotor changes to the CMPS-F. However, it also included physiological variables (appetite and blood pressure) combined with the dynamic, and interactive assessment. The scale was then translated into English and shown to be valid, reliable and responsive in the assessment of cats following ovariohysterectomy (32). A responsive scale is one which is sensitive to clinically relevant change. Which, in this case, are the expressions, psychomotor and physiologic changes associated with pain (24). Both the Portuguese and English versions have intervention scores of greater or equal to 8 of a possible 30. Additional translations to French and Spanish have been validated as well (36, 37). While this scale is perhaps better suited for the assessment of acute pain following ovariohysterectomy than the CMPS-F, it is limited in its application to other acute pain states. Additionally problematic is that as

a clinical tool the UNESP-MCPS is a more time-consuming and laborious (particularly the physiological variable collection) process. At present, the UNESP-MCPS is the only instrument shown to be qualitatively valid, reliable and sensitive (24). The inclusion of the grimace scale to the CMPS-F has improved the discriminatory ability of the rCMPS-F, however direct evaluation of criterion validity remains unreported. As a clinical tool, however, the rCMPS-F has been validated across a wider range of different pain types and sources (e.g. illness, surgery, emergency) making it, perhaps, more clinically applicable (34).

2. Nociceptive investigation

Quantifying pain is essential to its investigation. By providing objective measures of pain it becomes possible to compare different painful conditions in terms of diagnosis, treatment and progression. Pain is a perception, as such its quantification is difficult, particularly in non-verbal species. By providing a noxious stimulus and observing the response processed by the somatosensory system, it is possible to investigate thermal, electrical, mechanical, and/or chemical nociception (38). Regardless of its intended purpose, nociceptive investigation relies on the assumption that clinical pain correlates to the objective measure employed and the corresponding behavioural response. Limitations then exist due to anatomical, physiological and behavioural considerations, weakening this assumption. (39-42)

2.1 Quantitative sensory testing in people

Quantitative sensory testing (QST) is a widely used tool in human medicine for experimental and clinical research, as well as providing a bedside pain assessment (43, 44). QST assesses both static and dynamic measures of thermal and mechanical stimuli to evaluate the somatosensory system (44, 45). Investigators are capable of evaluating different peripheral nociceptive pathways and nerve types as well as spinothalamic and lemniscal central pathways by utilizing multiple stimuli in unison (46). In

general, thermal stimuli rely on A-delta and C nerve fibres and the spinothalamic pathway. Mechanical stimuli additionally involve A-beta fibres and the lemniscal pathway. The impact of painful conditions and treatments on pain transduction, transmission, perception and modulation can be made by comparing these various nerve fibres and pathways in conjunction. These stimuli test the nociceptive thresholds, limit of tolerance, as well as predefined parameters in which individuals' perception is evaluated. In non-verbal patients, QST is restricted to threshold testing as the expression of perception is limited. In these individuals the use of a proxy evaluator is required (47). A proxy evaluator interprets the response from an individual incapable of communicating their response directly. (43-46, 48)

2.2 Nociception models in animals

The inability of animals to communicate verbally limits the evaluation of pain to proxy observation (38). As is the case with non-verbal humans, nociceptive testing is thus limited to threshold testing for which a clear behavioural and motor response is elicited. For behavioural models of nociception, there are several requirements of an ideal nociceptive stimulus (38). First, it should be specific. Input specificity deems that the stimuli must induce nociception. Output specificity determines that the behavioural response must be preferentially or exclusively elicited by nociceptive stimuli. Next, the model should be sensitive. The response must be quantifiable, and the degree of response should correlate with the level of stimulus. A sensitive test should be capable of differentiating pharmacological (or other interventional) changes (eg. different drugs, dose, route of administration, etc.). The model should also be valid. In this sense, the test must allow easy differentiation between behavioural changes associated with nociceptive stimuli, and those that are non-specific or pharmacologically induced. The behavioural responses should not be invoked by sham testing. The model should be reliable. There must be consistency of the objective measure and repeated application should not result in lesions which may increase or decrease the response. This can be achieved by the

use of cut-offs, summation, or limiting duration of noxious stimuli. Finally, the nociceptive model must be repeatable; results must also be reproducible in repeated investigations.

2.3 Nociceptive tools in feline acute pain research

Mechanical threshold testing consists of the measurement of a force required to elicit a behavioural response. Von Frey apparatuses can measure this force directly (SI unit: newtons). Alternatively pressure can be measured as a surrogate for the mechanical force. This includes pressure-provoked threshold tools and pressure measurement mats. Finally, the response to palpation or orthopaedic manipulation where an observer subjectively determines whether a response is normal or abnormal. When using a pressure sensitive mat, two methods are used; peak vertical force measurement and pressure platform mat (24, 49). The assumption with mechanical thresholds is that when applied to painful areas (or when force is applied to a painful limb) the threshold will reduce compared to non-painful states. Additionally, the use of analgesics should increase threshold responses. Mechanical antinociception has been demonstrated following administration of opioids (morphine, buprenorphine, methadone, pethidine, and epidural hydromorphone), acepromazine, epidural tramadol, and alpha-2 agonists (medetomidine and dexmedetomidine) (50-56). Mechanical threshold testing is advantageous in its ability to detect painful responses. Validation has been performed on mechanical devices in cats (54). The problem with these devices is that there are a variety of available equipment and possible methods for mechanical testing, making comparisons of thresholds between studies problematic.

Electrical nociception is a stimulus which can easily be modulated and cut-off when the response is reached. It is limited in its utility as it is possible for the signal to stimulate multiple nerve types, thus reducing the nerve fibre specificity for nociception (57). Electrical stimuli are of limited use in conscious cats. However, they are often used in minimum alveolar concentration (MAC) studies (52,

58, 59). As a tool for investigating acute pain in conscious cats, electrical nociception is limited at this time (49).

Thermal nociception has been evaluated using thermal thresholds and radiant heat emitting mats. These both utilise a behavioural and motor response to escalating temperature to determine threshold response (24). The assumption with thermal testing is that analgesics will increase thresholds, while inflammation and/or painful states will reduce them. As a tool thermal nociceptive testing has been validated for use in cats for opioids (pethidine, buprenorphine, butorphanol, morphine, methadone) (51, 60-66). Thermal antinociception has also been demonstrated for tramadol and dexmedetomidine (61, 67, 68). Acepromazine did not alter thermal nociception despite increases in mechanical antinociception, this makes sense since acepromazine is not considered an analgesic (50). Like mechanical thresholds, in the absence of inflammation, thermal thresholds are insensitive to the use of NSAIDs (69). This limits the evaluation of NSAIDs with thermal threshold testing as it is a poor surrogate for inflammatory pain, a common post-operative pain source. Thermal antinociceptive testing is a validated tool capable of advanced drug comparisons particularly with opioid and opioid-like drugs.

Benefits to the use of thermal antinociception in cats is the ability to evaluate analgesic drugs in a minimally invasive manner with the absence of producing a chronic pain or diseased state. This tool is well tolerated by free-roaming cats, which minimises the influence of restraint on behavioural response to the noxious stimuli (60). For the investigation of opioids, the device has been used to compare drugs, evaluate dose-response relationships, route of administration comparisons, pharmacokinetic-pharmacodynamic modelling, and ultimately determine clinical efficacy (51, 60-66). The device has been most frequently used for drug comparison in cross-over studies. This is possible since an induced disease state is not a requirement for thermal antinociceptive testing. In these

comparisons, increased magnitude of thermal antinociception would suggest superior analgesic effect. Duration of thermal antinociception would be representative of different drugs' duration of analgesic effects. Presumably, if two opioids of the same class (eg. agonists of μ-opioid receptor) are administered at equipotent doses by the same route of administration, they should have an equivalent magnitude of thermal antinociception with variable duration of effect (51, 63). Dose-response relationships evaluate the same drug at different doses. This may be simply as a dose-finding study (68), a comparison of the effect of dose on antinociception (64), or evaluation of the impact of dose on pharmacokinetics (66). Comparing routes of administration allows for the evaluation of antinociception at equal or variable doses using the same drug. This would allow a better understanding of the impact of route on drug pharmacodynamics. This can be combined with blood sampling to determine pharmacokinetic variables and allow for pharmacokinetic-pharmacodynamic modelling (62, 65, 70). The final goal of all these experimental techniques is to determine clinical analgesia. Despite variation in thermal antinociception between studies, recent review articles have shown clinical dose intervals similar to some of these antinociceptive values (71, 72). In an attempt to refine clinical application of thermal antinociception, a recent article proposed that increases in mean thermal thresholds greater than two standard deviations above baseline thresholds may correlate with clinical analgesia (66). This approach appears to help diminish the impact of individual variability seen in this nociceptive model. Regardless of the method of evaluation or interpretation, thermal antinociceptive threshold testing provides a minimally invasive tool which allows for the evaluation of drug pharmacology and clinical analgesia (60).

3. Management of Acute Pain

The effective management and prevention of acute pain is essential to improve and maintain the welfare of veterinary patients. Appropriate pain management allows these animals to return to

normal function and prevent procedural or pain-associated morbidities. Balanced analgesia and multimodal anaesthesia are approaches which ideally target all aspects the pathway of pain (transduction, transmission, perception and modulation) simultaneously (73). Multimodal anaesthesia applies this principle to general anaesthesia whereby the combination of multiple therapies allows for reduction of individual treatment dosages (74). The presumption is that this will improve the beneficial effects of each drug while limiting their side- or adverse-effects. Balanced analgesia is the principle applied to the acute or post-operative period whereby combination therapies maximise analgesia while limiting the adverse-effects of, traditionally, single-agent opioid treatment. The foundation of a balanced, multimodal approach to developing an analgesic plan includes the use of; [1] Opioids, [2] NSAIDs, and [3] Local anaesthetics. For each class, there are considerations which may support or preclude the use, usually determined on a case-by-case basis. [4] Additional therapeutics may include agonists of the α_2-adrenergic receptor, antagonists of the *N*-methyl-*D*-aspartate (NMDA) receptors, gabapentin, tricyclic antidepressants, and/or anticonvulsants. And finally, [5] non-pharmaceutical management, which ranges from bedside (or, cage-side) nursing to complimentary treatments. While these techniques apply to all species, the following discussion will focus, when possible, on feline-specific management. (75, 76)

3.1 Opioids

At clinically effective doses, opioids are efficacious analgesics which can be used in a multitude of scenarios. As previously discussed, agonists of μ-opioid receptors have been historically avoided in cats due to the perceived risk of excitation or dysphoria (11). While favoured for use globally as the primary analgesic modality (with the exception of NSAIDs in the United Kingdom), limitations exist with some of the less potent opioids (e.g. buprenorphine, butorphanol) or opioid-like drugs (e.g. tramadol)

(17, 18, 21). These drugs have become popular due to their lower scheduling or perceived reduced adverse-effects, despite being less efficacious analgesics (13, 17, 18, 21).

The agonists of μ-opioid receptors commonly used in veterinary practice include morphine, hydromorphone, oxymorphone, pethidine (meperidine), fentanyl, remifentanil, and methadone. Morphine is regarded as the prototypical full agonist of μ-opioid receptors, to which all others are compared. The study of full-μ opioids in cats dates back to the beginning of the previous century (11). Initially, in comparative biomedical research, the doses used far exceeded current clinical doses (e.g. 5 mg kg^{-1}) (11). Studies exploring the pharmacology of doses which provide clinically relevant analgesia with opioids has occurred predominantly in the last two decades. Morphine (0.1-0.4 mg kg^{-1}, q 4-6 h, IV/IM/Epidural) is a full agonist at the μ, δ and ƙ opioid receptors (71, 75-77). Morphine has been shown to provide a MAC sparing effect. Initially it was reported to have a MAC sparing effect when given epidurally at 0.05–0.2 mg kg^{-1} (maximum reduction 30.8 ± 9.6%) (78). A more recent study could not reproduce this MAC reduction from epidurally administered morphine (79). The MAC sparing effect of IV morphine was demonstrated in cats with isoflurane at 1 mg kg^{1} (maximum reduction, 28 ± 9% at 180 min) however, at 0.1 mg kg^{-1} a reduction (maximum reduction, 12 ± 4% at 60 minutes) was not clinically relevant (80). These three studies evaluated MAC using a tail-clamp technique making stepwise 10% increases or decreases in anaesthetic concentration dependent on whether or not there was purposeful movement. In the IV study, a ten-fold difference in dose was evaluated, it would be interesting to see if there is a MAC reduction at intermediate doses than those reported. This binary evaluation of two morphine doses does not preclude the possibility of a dose-dependent MAC sparing effect of morphine. Like other species, there is a risk of histamine release in cats when morphine is given IV which may result in vasodilation, hypotension, and/or hypersensitivity reactions. Histamine release is dose-dependent, when given slowly at lower doses it can be minimized. As already discussed,

the active metabolite, morphine-6-glucuronide is limited in its production in cats (10). As a result, the drug might not be as efficacious in cats as in other species. Despite this fact, morphine has been shown to increase TT in cats (63). Although widely available, morphine is not licensed for use in veterinary species. Hydromorphone (0.025-0.1 mg kg^{-1}, q 4-6 h, IV/IM) is a semi-synthetic opioid that is used across North America (71, 75-77). It is a more potent agonist of μ-opioid receptors compared to morphine. Hyperthermia is an adverse effect seen frequently (up to 69%) and is specific to cats (81). Typically conservative management (active cooling with fans, cold packs, etc.) is sufficient in treating the hyperthermia. However, in the same study, 2 out of 49 cats required naloxone treatment to correct their hyperthermia (when temperature was > 41.6 °C). Oxymorphone (0.025-0.1 mg kg^{-1}, q 4-6 h, IV/IM) is similar to hydromorphone in terms of potency, however it is not associated with vomiting or hyperthermia (76, 82, 83). Like hydromorphone, it is commonly used in North America, but hydromorphone is a more cost-effective analgesic than oxymorphone (84). Pethidine (3-5 mg kg^{-1}, q 1-2 h, IM) is a synthetic agonist of μ-opioid receptors (71, 76, 77). It is licenced, and more commonly used, in the United Kingdom, and parts of Europe for cats (3.3 mg kg^{-1}, IM) (71). Its use is limited to IM injection, due to IV histamine release. It is an agonist of μ-opioid receptors which rarely causes vomiting. The drug has a rapid onset (approximately 30 minutes) and short duration of action (1 to 2 hours), limiting its clinical utility. Fentanyl (Bolus 1-10 μg kg^{-1}, IV; CRI 2-20 μg kg^{-1} h^{-1}, IV; Patch 25 μg $hour^{-1}$, adult > 4 kg) is a potent, short-acting synthetic agonist of μ-opioid receptors (71, 75-77). Fentanyl is not licensed for use in animals, in addition to being a scheduled (Canada schedule I, United Kingdom schedule II) drug. Scheduled drugs are those which are controlled by federal governments based on their likelihood for abuse and medical usage. In general schedule I drugs are the most controlled, with the highest likelihood for abuse and often limited medical usage. Subsequent classifications are less controlled. Scheduling and degree of control varies depending on country/governing body (85). Returning to fentanyl, the drug has been shown to be efficacious in

conjunction with either inhaled or intravenous anaesthetics (86). When used as a patch for cats, the effects are individually variable, and absorption is less consistent than in dogs. Pain assessment and multimodal analgesia is recommended when using transdermal patches. Remifentanil (CRI 4-18 μg kg^{-1} h^{-1}, IV) is used intraoperatively and is similar to fentanyl (71, 75-77). It is shorter-acting and does not require hepatic metabolism (87). This latter fact may be beneficial to not only cats with hepatic disease, but also healthy cats due to their unique drug metabolism (9, 10). Remifentanil also has a comparatively shorter context-sensitive half-life (time to reduce plasma drug concentrations by half following termination of a continuous rate infusion) compared with fentanyl (88). This allows the drug to be more rapidly titrated to effect. However, stopping the infusion requires the absence of noxious stimuli or alternative pain-relieving treatments as the analgesic effect of remifentanil is lost rapidly. Finally methadone (0.3-0.6 mg kg^{-1}, q 4 h, IV/IM/OTM) is a synthetic agonist of μ-opioid receptors with NMDA receptor antagonism (71, 75-77). It is licensed for use in the United Kingdom, Italy, and a few other countries in Europe for cats (71). Its additional mechanisms of action show promise in the management of hyperalgesia, although future study is required. (12, 71, 75-77, 89, 90)

Buprenorphine (0.01-0.24 mg kg^{-1}, q 4-24 h, IV/IM/SC/OTM) is a highly potent, semisynthetic partial agonist of μ-opioid receptors (71, 72, 75, 77). Depending on the species it may have κ antagonism or even μ antagonism (at high doses) (62, 91). At low doses (0.01-0.04 mg kg^{-1}) with low concentration formulations (0.3 mg ml^{-1}), buprenorphine has been licenced widely across the USA and Europe (71). At these low doses, SC administration of the drug is ineffective compared to the IV/IM/OTM routes of administration in terms of drug absorption and analgesic effect (65). This resulted in the recommendation that buprenorphine should not be administered by the SC route of administration (65, 72, 77). However, more recently, there has been an interest in higher doses and increasing concentrations of buprenorphine, these high doses have demonstrated prolonged (> 24 h)

thermal antinociception compared to low-dose IV/IM/OTM buprenorphine administration (66). Recently a high-concentration formulation of buprenorphine (1.8 mg ml^{-1}; Simbadol, Zoetis, NJ, USA) has been licensed for use in the United States (0.24 mg kg^{-1}, q 24 h, SC) perioperatively for 3 days. There is an absence of information regarding the pharmacokinetics and pharmacodynamics of Simbadol, as well as the antinociceptive properties when it is given by other routes of administration. Buprenorphine can be used for mild to moderate pain, typically combined with a sedative (65, 67). Studies in cats have supported its use for more invasive procedures, such as ovariohysterectomy when combined with an NSAID and local anaesthesia (92). Buprenorphine has a stronger affinity for the μ-opioid receptor compared to morphine and other full agonists of μ-opioid receptors. This is problematic when dealing with breakthrough pain in patients managed with buprenorphine, as the simple addition of a more potent (in terms of analgesic efficacy) full agonist of μ-opioid receptors may be ineffective as buprenorphine's strong receptor affinity prevents displacement with the alternative opioid (93). One study showed thermal antinociception was influenced by administration of buprenorphine (0.02 mg kg^{-1}, IV) 30 minutes after hydromorphone (0.1 mg kg^{-1}, IV), however, it was unclear the impact on magnitude or duration of effect (93). Without further investigation, alternative modalities may be required until the end of buprenorphine's duration of action when it is combined with other opioids. (71, 72, 77)

Butorphanol (0.1-0.4 mg kg^{-1}, q 1-2 h, IV/IM) is a synthetic opioid with mixed agonist/antagonist activity (71, 75-77). Like buprenorphine, its affinity and activity is species-specific for the opioid receptor subtypes. Butorphanol's duration of action is shorter than buprenorphine (71, 76, 94). For cats undergoing ovariohysterectomy, the drug's analgesic efficacy is less than that of buprenorphine (95). When butorphanol or buprenorphine are combined with an NSAID post-operative analgesia is similar (96). Butorphanol is a poor somatic analgesic (analgesia to the skin, superficial, and deep tissues),

though it does appear to provide effective visceral analgesia (analgesia of the internal organs) (12). Unlike full agonists of μ-opioid receptors, there is a "ceiling" effect owed to the agonist-antagonist mechanism, limiting its analgesic effect (94). Butorphanol provides a reliable sedation and has MAC-sparing effects (80). It also is a potent antitussive (97). As it is a μ-antagonist, it can also be used as an antagonist of drugs which are agonists of the μ-opioid receptor in the case of dysphoria, cardiorespiratory depression or excessive sedation (e.g. with oxymorphone) (98). For cats, butorphanol has an important role for sedation and premedication for less invasive procedures. (12, 71, 75, 77, 97)

In the clinical setting, there is only one opioid-like drug, tramadol (2-4 mg kg^{-1}, q 6-8 h, IV/IM/PO) in use (71, 75-77). Tramadol is not licensed for use in cats, however it is licensed in a few European countries for dogs (71). It is a scheduled drug, III in the United Kingdom and IV in the USA (71). Despite this, the drug is widely used owing to its available oral formulation (in North America) and injectable form (South America and Europe). Palatability when given orally has been identified as being problematic (99). Oral formulation allows for continued analgesic treatment at home, potentially shortening hospitalization. Tramadol has been evaluated in a number of clinical studies. As with buprenorphine and butorphanol, its use as a single-agent analgesic is likely insufficient for painful procedures or conditions (77). When used in conjunction with NSAIDs or other modalities, it has proven to be beneficial (100, 101). Tramadol's mechanism of action depends on the activation of the μ-opioid receptor as well as serotonin and norepinephrine reuptake inhibition. The drug does require metabolism for activation, which does not occur equally and reliably in all patients. Cats can produce the active metabolite, O-desmethyl-tramadol. Metabolism produces optical isomers. The (+) metabolite has increased affinity for the μ-opioid receptor and causes serotonin reuptake inhibition (102). The (-) isomer is responsible for norepinephrine reuptake inhibition. The production of this (-) isomer may be limited in cats. Individual variability in metabolism may be responsible for the

inconsistent analgesic effect (77, 101). Tramadol has been suggested to be a superior analgesic to pethidine, however this conclusion was based on its longer duration of action (2 h vs 7 h) following ovariohysterectomy in cats (103). There are also early studies showing a potential benefit to the use of tramadol in chronic pain (e.g. osteoarthritis) in cats, however further investigation is warranted. (71, 75, 77, 100, 103)

Tapentadol is a novel atypical opioid drug with a dual mechanism of action. The drug is an agonist of μ-opioid receptors and inhibits nor-epinephrine re-uptake (104). Unlike tramadol, tapentadol is administered in the active form and does not require hepatic metabolism for an analgesic effect. Tapentadol is metabolized by hepatic glucuronidation in people by uridine 5'-diphospho-glucuronosyl transferases (UGTs) (105, 106). Tapentadol does not induce or inhibit hepatic cytochrome P450 enzymes (106). This is considered to be an advantage, as it can be administered to patients with mild to moderate renal or hepatic impairment (107). The efficacy of tapentadol is similar to that of morphine in the treatment of acute and chronic pain in humans (108). The similar efficacy highlights the importance of the nor-epinephrine re-uptake inhibition pathway since tapentadol has 18-fold lower affinity for the μ-opioid receptor in people compared to morphine. In veterinary medicine, many studies have been published on the pharmacokinetics of tapentadol in different species (109-111). In dogs and cats, the pharmacokinetics of tapentadol after parenteral administration have been described (112, 113). Bioavailability of tapentadol is low after oral administration at 4.4%, 9% and 32% in dogs, rats and humans, respectively (112, 114). Tapentadol has been shown to have antinociceptive effects in dogs (111). The analgesic and antinociceptive effects of tapentadol are unknown in cats.

Opioids have additional effects beyond their analgesic and sedative qualities. Some qualities appear to be positive, these include, playfulness, purring, rolling, and kneading (i.e. euphoria) (71). Others may have more undesirable effects. The most important adverse effects of the opioids are

bradycardia and respiratory depression in conscious, sedated and anaesthetised patients (71). Monitoring patients receiving these drugs is important. Morphine and hydromorphone are associated with salivation and vomiting in cats and dogs (71). These adverse effects are reported in antinociceptive and clinical studies, despite not being the primary focus of investigation. Ileus following opioid administration appears to be limited following single-doses in healthy individuals (76). Monitoring gastric transit following any procedure or continued opioid use is recommended. Opioids cause mydriasis in the cat, this has deleterious effects on visual acuity and depth perception, which may cause individuals to walk into objects or startle to approaching handlers. Hyperthermia and transient increases in body temperature have been seen with buprenorphine, butorphanol, morphine, hydromorphone and alfentanil. Monitoring body temperature following the use of opioids is recommended. Emerging topics in opioid side effects and responses include pharmacogenomics and hyperalgesia/tolerance. Pharmacogenomics attempts to account for the variation in response to opioids and takes a genotypic and phenotypic approach to formulating an analgesic plan for an individual (maximizing analgesia and minimizing side-effects) (77). Hyperalgesia and tolerance are seen in human medicine, however the incidence in veterinary species is unknown. (71, 76, 77)

3.2 Nonsteroidal anti-inflammatory drugs

NSAIDs are commonly used in cats for the prevention or treatment of acute pain associated with surgery, trauma or disease. Both therapeutic and toxic effects are produced by the inhibition of cyclooxygenase (COX) and alterations in prostaglandin production (115). An ideal NSAID should spare COX-1 and inhibit COX-2 to a degree for anti-inflammation and analgesia, but not entirely to interfere with normal physiologic processes (116). Normal COX-1 function is vital for the protection of gastric mucosal, platelet function and renal perfusion. Inhibition of this isoform would be deleterious to the gastrointestinal system, coagulation, and renal function (115). COX-2 is involved in inflammation and

nociception as well as normal function of the renal, nervous, and reproductive systems. Historically, NSAIDs were non-selective for COX isoforms. More recently emphasis has been placed on COX-2 selectivity. While this has minimised toxicity, potential adverse effects remain (117). The ideal NSAID should target sites of inflammation to minimise these adverse effects and be rapidly cleared from the central compartment (116). This effect is due to the protein binding of NSAIDs, which is normally high. When the drug is delivered to the inflamed site, pH becomes more acidic. If a drug then has a low pK_a, it will dissociate. In this active state, the drug can freely enter cells and remain in the target site (116). (75, 76, 115-117)

A wide variety of NSAIDs are marketed and available for use in veterinary medicine. For dogs, this availability is greater than cats. The NSAIDs commonly used for feline patients include meloxicam, robenacoxib, carprofen, ketoprofen and tolfenamic acid (117, 118). These are the approved products for acute pain in cats in the United Kingdom. In Canada, all but carprofen is licensed as well. In the USA only meloxicam and robenacoxib are licensed. The provided doses are those approved for use in Canada, where applicable. Meloxicam (0.05-0.2 mg kg^{-1}, q 24 h, SC/PO) is a COX-2 preferential NSAID (117-119). It is considered to be tissue-selective as a function of the mechanism described above (116). Following oral administration, meloxicam is principally metabolised by oxidation and excreted fecally (120). as well as being an effective analgesic in the management of Robenacoxib (1-2.4 mg kg^{-1}, q 24 h, SC/PO) is a highly selective COX-2 inhibitor (117-119). It has a similar inflammatory tissue persistence and faster central compartment clearance when compared with meloxicam (116). *In vivo* meloxicam was shown to inhibit COX-1 for up to 24 hours, whereas robenacoxib did not (121). Despite these superior qualities, benefits of isoform selectivity and tissue persistence have not been shown in the cat (116). Carprofen (4 mg kg^{-1}, SC/IV, not approved in Canada) is a COX-2 preferential NSAID only licensed for single-dose administration. Finally both ketoprofen (2 mg kg^{-1}, q 24 h, SC/PO) and tolfenamic acid

(4 mg kg^{-1}, q 24 h, SC/PO) are COX inhibitors which show no isoform selectivity (117-119). NSAIDs have been shown to be effective in the treatment of pyrexia in cats (118, 122). Their analgesic efficacy has been demonstrated across a wide range of procedures including ovariohysterectomy, castration, orthopaedic and other soft tissue surgeries (82, 116-119, 121, 123-127)

3.3 Local Anaesthetics

Local anaesthesia is the sole modality capable of complete analgesia (76). Local anaesthetics interrupt conduction and propagation of nerve action potentials via binding of voltage-gated sodium channels. In addition to being effective, these methods are typically inexpensive and associated with minimal complications (128). Local anaesthetic techniques can range in difficulty from simple, such as for topical or infiltrative administrations, to advanced, where techniques may require advanced imaging modalities (75). Onset of action and duration of analgesia varies depending on the drug used. The following paragraph includes local anaesthetics commonly used in feline practice. The maximum dose is provided, as clinical dosage often depends on the technique utilised. A description of all the available techniques is beyond the scope of discussion of this review. In general, a strong knowledge of neuroanatomy and techniques allows practitioners the ability to either block nociception at the site of interest, or more proximally, preventing afferent nerve conduction. (75, 76, 128, 129)

Lidocaine (maximum recommended dose, 6 mg kg^{-1}) is a fast-onset (5-10 minutes) and medium duration (1-3 hours) local anaesthetic (75). In other species, lidocaine may be used as an IV bolus or perfusion producing analgesia and sedative effects. Despite a significant reduction in MAC when used IV, lidocaine causes increased cardiovascular depression than an equivalent concentration of isoflurane in cats (130). Its use in this manner in cats is not recommended. Bupivacaine (maximum recommended dose, 2 mg kg^{-1}) has a slow onset (10-20 minutes) and long duration of action (3-6 hours). Mepivacaine (maximum recommended dose, 3 mg kg^{-1}) has an onset (3-10 minutes) similar to lidocaine and a

duration intermediate to the two previous (2-4 hours). Ropivacaine (maximum recommended dose, 1.5 mg kg^{-1}) has a similar profile for onset (15-20 minutes) and duration (1.5-6 hours) to bupivacaine, however, it is less potent. When used at lower concentrations, there is some evidence that ropivacaine may preferentially block sensory nerves and not motor nerves (131). This mechanism is not fully understood but possibly involves the drug's lower lipid solubility limiting penetration of larger A-beta fibres. All of the drugs described are amide local anaesthetics, these are typically metabolised by hydroxylation, dealkylation, and methylation then excreted by the liver (75, 131, 132).

3.4 Additional therapeutics

There are many remaining adjunctive drugs and classes of drugs which are used for the management of acute pain. Two that are commonly used with direct analgesic intent include ketamine and the α-2 receptor agonists. While these drugs have a direct role in antinociception and analgesia, when used as sole-agents their analgesia is limited. As a result, when used, these drugs should be combined with more efficacious analgesics.

Ketamine (0.5-20 mg kg^{-1}, IV/IM or as a perfusion 1-30 μg kg^{-1} min^{-1}) is a NMDA receptor antagonist capable of achieving a dissociative anaesthesia state and providing antihyperalgesic effect (75, 76). These effects are achieved via antagonism of NMDA receptors and non-NMDA glutamate receptors, agonism of μ-, κ-, and δ-opioid receptors (in reducing affinity) ultimately resulting in reduced central nervous sensitization and increasing inhibitory nerve activity (133). More recently elucidated mechanisms, in human medicine, include opioid potentiation, anti-inflammatory, anti-proinflammatory, and antitumour effects (134). The clinical use of ketamine is limited due to its controlled status and the parenteral administration. Ketamine is most frequently used in feline practice in combination with diazepam ("Ket-Val") or as an intramuscular injection combined with a sedative and opioid. A recent study showed significant reduction in isoflurane requirements associated with

ketamine (0.5 mg kg^{-1} bolus, and 30 μg kg^{-1} min^{-1}) in combination with remifentanil (20 μg kg^{-1} hr^{-1}) in cats undergoing ovariohysterectomy (135). Given its analgesic characteristics, continued use in this manner is supported.

Agonists of α-2 adrenoreceptors are most frequently used for their sedative qualities, which work synergistically with opioids (75, 76, 136). These combinations are useful for chemical restraint or peri-operative analgesia. The analgesic qualities of α-2's effect pain transmission, modulation and perception (137). The most commonly used α-2 agonists in feline practice are dexmedetomidine, medetomidine and xylazine, listed in decreasing α-2 receptor specificity. Dexmedetomidine (0.001-0.04 mg kg^{-1}, IV/IM) and medetomidine (0.001-0.150 mg kg^{-1}, IV/IM) are typically used for sedation and restraint (75, 76, 119). Dexmedetomidine has replaced medetomidine in most countries as the α-2 agonist of choice for this use. Xylazine (0.1-0.5 mg kg^{-1}, IV/IM) remains licensed in Canada for use in dogs and cats for premedication (119, 136). It is more commonly used in the emergency setting to induce vomiting in cats. Alpha-2 agonists provide analgesic effects which may benefit animals in the acute setting, however, this effect is often shorter than the sedation produced by the drug. Atipamezole, and α-2 receptor antagonist, allows for reversal of the α-2 receptor agonists, providing added security to this class of drug (136). The deleterious effects on the cardiorespiratory system, profound sedation, and parenteral administration limit the use of α-2's to the clinical setting. MK-467 is a peripheral α-2 receptor antagonist, which has been given in combination with this class of drugs to mitigate the impact of α-2 agonists on the cardiovascular system (138). Their inclusion appears to diminish the analgesic effects of α-2 receptor agonists.

3.5 Non-pharmaceutical management

Often pharmacologic therapy is considered alone when producing an analgesic plan. Enhanced perioperative care and comfort includes limiting stress, gentle handling, and provision of warm and comfortable enclosures (75). Fluid management should be tailored to the individual, and in some instances requires pre- and post-operative therapy. Hydration and nutrition are also important in optimising healing and recovery. While these features are usually addressed in the course of surgical interventions, they are rarely tailored to the procedure, health status, age, and anticipated recovery period of the animal. In human medicine, this field, enhanced recovery programmes is extensively researched (139). Recovery plans are aimed at providing preoperative (patient interview and education, premedication selection, and determination of optimal nutrition), intraoperative (surgical technique, anaesthetic protocol), and post-operative (analgesic plan, nutrition, and rehabilitation) guidelines which are intended to reduce hospital stay, optimize comfort, and improve quality of life. While these principles can be translated to veterinary medicine, similar investigations should be undertaken to assess the impact of these variables on patient-outcome directed goals.

Specific non-pharmaceutical interventions have been shown to be beneficial in controlling pain. Cold therapy is an effective and inexpensive treatment for acute inflammatory pain. Cold retards cellular metabolism, and subsequently tissue damage. It also produces vasoconstriction, reducing oedema. Cold therapy can decrease nerve conduction, decreasing transmission of noxious stimuli (140). Heat has the opposite effect to cold at the cellular level. This increases circulation and cellular metabolism, which is beneficial in tissue healing. Heat also improves mobility, which in conjunction with physical rehabilitation can improve range-of-motion and return-to-function (140). Other physiotherapy techniques include stretching, massage and exercise. These techniques function similarly to heat by improving circulation and improving oxygenation and recovery. Acupuncture and

electroacupuncture are additional complimentary analgesic modalities. There are limited prospective, controlled and randomised studies available for acupuncture in cats. What is available shows increasing evidence supporting the mechanism of action for the technique. Needle placement is associated with the endogenous release of endorphins, enkephalins, and serotonin all of which produces analgesia. In both acute and chronic models of pain, beneficial effects have been seen in dogs (140-142). One study investigating laser acupuncture in cats undergoing ovariohysterectomy demonstrated a positive effect on patient comfort post-operatively (143). As an adjunct to multimodal analgesia, acupuncture may prove beneficial in the acute-pain setting. Additional ancillary techniques include transcutaneous electrical nerve stimulation, extracorporeal shockwave therapy, stem cell therapy, platelet-rich plasma, polysulfated glycosaminoglycans, and other nutraceuticals. These techniques are often utilised in the management of chronic pain. It is possible, however, their use following surgical intervention may be beneficial acutely by limiting or preventing morbidities. Further investigation of these techniques are warranted as their use cannot be supported or refuted based on the available literature. (75, 76, 140, 143)

4. Novel investigation

There is a need to investigate novel analgesics and treatments for the management of acute pain in cats. As previously discussed, historical misconceptions, unique metabolism and restricted drug access has limited analgesic availability in feline practice. Experiments using novel drugs or current drugs in a new manner need be investigated. The use of thermal threshold testing allows for a minimally invasive evaluation of nociception in this species.

4.1 Objectives

The objective of this thesis was to evaluate novel analgesic therapeutics for use in cats.

First there was an evaluation of the impact of route of administration on the pharmacokinetic and pharmacodynamics of a high-concentration formulation of buprenorphine using thermal antinociception in conscious cats.

Then a novel drug, tapentadol, was evaluated after oral administration in a dose-finding study using thermal antinociceptive testing.

Article 1

Pharmacokinetic and pharmacodynamic modelling of subcutaneous, intravenous and buccal high-concentration buprenorphine in conscious cats

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Abstract

Background

The aim of this study was to describe the joint pharmacokinetic-pharmacodynamic model and evaluate thermal antinociception of a high-concentration formulation of buprenorphine (Simbadol™) in cats.

Methods

Six healthy cats (4.9 ± 0.7 kg) were included in a prospective, randomized, blinded, crossover study. Simbadol™ (1.8 mg mL^{-1}) was administered by the subcutaneous (SC; 0.24 mg kg^{-1}), intravenous (IV; 0.12 mg kg^{-1}) or buccal (OTM; 0.12 mg kg^{-1}) route of administration and thermal thresholds (TT) were compared with a saline group (SAL). Thermal threshold testing and blood sampling were performed at predetermined time points up to 72 hours including a placebo group. Plasma buprenorphine and norbuprenorphine concentrations were measured using liquid chromatography mass spectrometry. A bespoke bicompartmental pharmacokinetic model simultaneously fitted data from two analytes/three routes of administration. Temporal changes in TT were analyzed using one-way ANOVA followed by Dunnett's test and treatment comparisons using two-way ANOVA with Bonferroni's correction ($P < 0.05$).

Results

Thermal thresholds were significantly increased after SC, IV and OTM from 1-24 hours (except 2 hours), 0.5-8 hours (except 6 hours), and 1-8 hours (except 6 hours), respectively, when compared with baseline. Thermal thresholds were significantly increased after SC (1-30 hours), IV (1-8 hours) and OTM (1-12 hours) when compared with SAL, but not different among buprenorphine-treated cats. The absolute buprenorphine clearance was 0.98 L kg^{-1} $hour^{-1}$, volume of distribution at steady

state was 7.9 L kg^{-1} and the elimination-half-life was 12.3 hours. Bioavailability for SC and OTM was 94% and 24%, respectively. Subcutaneous absorption was biphasic. An initial peak (0.08 hours) was followed by a slow (half-life 11.2 hours) and progressive (peak acceleration at 2.8 hours) uptake.

Conclusion

The SC administration of Simbadol™ was characterized by prolonged absorption half-life and sustained plasma concentrations yielding long-lasting antinociception (≥ 24 hours) when compared with the IV and OTM routes.

Introduction

Buprenorphine is an opioid analgesic drug that is commonly administered for the treatment of feline perioperative pain. The use of buprenorphine in this species has been recently reviewed in both experimental and clinical setting (72). At standard clinical doses (0.02 mg kg^{-1}) and concentrations (0.3 mg mL^{-1}), buprenorphine is poorly absorbed and has limited antinociceptive effect after subcutaneous (SC) administration (65). Further investigation showed that increased doses (> 1.2 mg kg^{-1}) administered by this route of administration can provide prolonged antinociception and improved absorption compared with standard doses (66). Simbadol™ (1.8 mg mL^{-1}, buprenorphine hydrochloride; Zoetis, NJ, USA) is a FDA-approved high-concentration formulation of buprenorphine for cats. The drug is indicated for the control of postoperative pain and approved for subcutaneous only administration at 0.24 mg kg^{-1} every 24 hours up to three days. There is an interest in investigating the antinociceptive effects, pharmacokinetics (PK) and pharmacodynamics (PD) of this high-concentration of buprenorphine after SC, buccal or intravenous (IV) administration in conscious cats. . Using a joint PK model of the three routes of administration would provide more robust estimates of the PK parameters of Simbadol™ when compared with traditional PK methods analyzing each route of administration separately (144).

The aims of this study were to 1) describe a joint PK modelling of Simbadol™ in awake cats, 2) evaluate the time-course of thermal antinociception in the same individuals, and 3) estimate PD parameters by PK-PD modeling. It was hypothesized that 1) joint PK would provide a robust method for understanding PK modeling, 2) Simbadol™ would produce dose-dependent thermal antinociception and 3) PK-PD modeling would explain how plasma concentrations of buprenorphine and thermal antinociception are correlated after administration of Simbadol™. This study reports joint PK modelling and PK-PD modelling of buprenorphine after the administration of Simbadol™.

Materials and Methods

Animals

The animal care committee of the Université de Montréal approved the study protocol (14-Rech-1761). This study is reported according to the ARRIVE guidelines (145).

Six healthy adult domestic short haired cats (4.9 ± 0.7 kg, four males and two females) were included in the study. The cats were group housed in a long-term accommodation (room) with temperature (20-22 °C) and humidity (40-70%) control, and according to the Canadian Council for Animal Care guidelines. The cats were fed a commercially available diet twice daily with *ad libitum* water. All cats were healthy based on physical examination, hematology and serum chemistry profile. Environmental enrichment was provided following the American Association of Feline Practitioners and International Society of Feline Medicine guidelines (146). During testing, cats were housed individually in stainless steel adjacent cages (67 x 55 x 68 cm^3). Body weight was monitored on a weekly basis. Cats were acclimated to the testing procedures several weeks before the study began (S1 - Appendix 1).

Experimental design

The experimental phase was divided into two parts.

Phase I (Thermal thresholds after saline 0.9%) - Cats were administered sterile saline 0.9% (NaCl 0.9%; Baxter, ON, Canada) by the subcutaneous route of administration (same volume as 0.24 mg kg^{-1} dose of Simbadol™) between the shoulder blades, and thermal thresholds (TT) were evaluated for up to 72 hours (see time points below). Blood collection or venous catheterization was not performed on Phase I to avoid unnecessary stress to the cats.

Phase II (Cross-over thermal threshold testing and blood sampling) - Phase II used a randomized, prospective, blinded, crossover study design with a 14-day minimum wash-out period between treatments. Approximately 12 hours before TT testing, a short-term catheter was introduced in the

cephalic vein and general anesthesia was induced and maintained using 8 mg/kg of propofol (Diprivan; AstraZeneca, ON, Canada) and isoflurane (Isoflurane, Aerrane; Baxter, ON, Canada) in 100% oxygen, respectively. A central venous catheter (Peel Away Single Lumen 19Ga, PI-1910; Mila International, KY, USA) was aseptically introduced in the jugular vein, sutured, and covered with light soft bandage. The jugular catheter was used for *blood sampling* throughout the study and removed after the testing period. The cephalic catheter was maintained in all cats until *treatment administration* and was used for administration of the test drug by the IV route when applicable. It was removed immediately after treatment administration. At the end of the procedure, cats were allowed to recover from anesthesia in a calm and quiet environment.

Cats were randomly assigned to receive one of the following treatments: a. Simbadol™ by the intravenous route (IV, 0.12 mg kg^{-1}) via cephalic catheter followed by flush with saline; b. Simbadol™ by the subcutaneous route (SC, 0.24 mg kg^{-1}). The drug was injected between the shoulder blades; and c. Simbadol™ by the buccal route (OTM, 0.12 mg kg^{-1}). The volume of buprenorphine was slowly administered into the cheek pouch using a 1mL syringe in the contralateral side to the central venous catheter position. Doses for the IV and OTM routes were based on previous safety and efficacy studies performed by the manufacturer, and clinical interest. Randomization was performed using an online software (www.randomization.com). Randomization and treatment administration were performed by two individuals who were not involved with TT testing (PS/GD).

Measurements of thermal threshold

Antinociception was evaluated using a wireless TT device (WTT1, Topcat Metrology Ltd, UK). The test has been validated in free-ranging cats (60) and several studies using the TT device and evaluating the antinociceptive effects of buprenorphine have been reported in the species (62, 63, 66, 67, 70, 81, 147-150). The equipment was calibrated and maintained according to the manufacturer's

recommendations. The TT device is incorporated into an elasticated vest, containing a wireless receiver, power supply, LCD display and thermal probe which is applied around the thorax of a free roaming cat. An adjustable air bladder is used to maintain consistent pressure and direct contact of the temperature probe with the shaved lateral thorax. For each measurement, the skin temperature (ST) is recorded prior to thermal stimulus. For TT testing, the evaluator triggers a ramped heat stimulus (0.6 °C second^{-1}) using a hand-held device which is stopped once the cat exhibits a behavioral response (e.g. vocalization, rolling, jumping, etc.; considered the TT), or when the cut-off of 55 °C is reached. If cut-off was reached, this value (55 °C_ was used as the TT. A single evaluator (BM) performed TT testing and was blinded to the treatments. Thermal threshold testing was evaluated before (baseline; time 0) and at 0.5, 1, 2, 4, 6, 8, 12, 24, 30, 36, 48, 60 and 72 hours after treatment administration for both phases. At each testing day and 30 minutes after placement of TT device, baseline values were determined using the mean of three recordings performed at 15-minute intervals prior to treatment.

Any behavioral changes, adverse reactions or additional observations were recorded during the testing period. Oral pH was measured at baseline (pre-treatment) and at 48 hours after treatment.

Blood sampling

During Phase II, blood sampling was performed from the jugular vein via central venous catheter after 2 and 8 min of treatment administration, and after each TT testing. The volume of blood collected was adjusted for each individual so that less than 10% of the cat's total blood volume was removed over the study period (2 mL maximum per sample). After each collection, the catheter was flushed with 1 mL of heparinized saline and the injection plug was changed to minimize contamination of subsequent sampling. Blood was transferred to K_3EDTA tubes and centrifuged at 2000 *g* for 10 minutes. Plasma was separated and stored at -80 °C before buprenorphine/ norbuprenorphine HPLC-MS/MS analysis.

Buprenorphine and norbuprenorphine assay

Appendix 2 (S2) provides description of the analytical method for buprenorphine and norbuprenorphine using high performance liquid chromatography-mass spectrometry (HPLC-MS/MS). The method met standards for sensitivity, linearity, precision, accuracy and stability generally accepted in bioanalytical chemistry (151). Limits of quantification were 0.1 to 100 ng mL^{-1} for buprenorphine and 0.2 to 100 ng mL^{-1} for norbuprenorphine.

Joint PK and PK-PD modeling

Population pharmacokinetic modelling was performed with Phoenix NMLE®, version 1.3, Certara (Princeton, NJ, USA) installed on a Dell Precision 7510 computer (core i7). Full description of the joint population PK and PK-PD model is provided in Appendix 3 (S3). Briefly, a two compartmental model was used to simultaneously model the plasma concentration-time curves of buprenorphine and norbuprenorphine (formed through conversion from buprenorphine and first pass effect). The SC, IV and OTM administration routes were included jointly in the PK model to increase the number of degree of freedom. In order to explore the biphasic nature of the SC absorption, several complex candidate models were elaborated and compared. Estimates of PK variables were provided with inter-individual variability (IIV%). Population PD parameters were estimated by sequential population PK-PD modeling. The best PD model was selected among a series of candidate models including negative hysteresis and antinociceptive effects of buprenorphine with or without norbuprenorphine.

Statistical analysis

Prospective power analysis concluded that a sample size of six cats would be sufficient to detect mean temperature differences of > 3.2 °C with a power of 0.8 and an alpha level set at 0.05 based on a previous study (152). Statistical analyses were performed using a software (GraphPad Prism, GraphPad software Inc., California, USA). Thermal threshold values were used as the outcome variable for

comparisons. Thermal thresholds for each treatment were analyzed for temporal changes using one-way ANOVA for repeated measures followed by the Dunnett's test when appropriate. Treatment comparisons were made using two-way ANOVA followed by Bonferroni's correction ($p < 0.05$). Data were expressed as mean ± SD.

Results

Adverse effects and behavioral changes - Adverse effects were not observed in Phase I. Overall, signs of euphoria (rolling, kneading with thoracic paws, meowing, and purring) and agitation were recorded after treatment with all routes/cats in Phase II. Vomiting and diarrhea were observed in two cats during the study; one in the IV group (diarrhea on day 2 and vomiting once on day 3) and one in the OTM group (diarrhea only on day 3). Both cats remained bright and alert and their clinical signs spontaneously resolved. Short-term hypersalivation was noted in two cats immediately following OTM treatment. Dysphoria was not observed in this study. Mean ± SD of oral pH before and after (48 hours) all treatments were 8.8 ± 0.4 and 8.7 ± 0.5, respectively.

Skin temperature and thermal thresholds - Mean ± SD baseline ST for all treatments (Phase I and II) was 36.9 ± 0.6°C. Skin temperature (ST) was not significantly increased after IV, OTM or saline 0.9% treatments when compared with baseline. Skin temperature was significantly increased in SC treatment between 60 and 720 minutes (except 120 and 360 minutes; $p = 0.0001$). These values were within normal range and hyperthermia (ST > 39.5 °C) was not observed. Skin temperature was significantly increased from 0 to 3600 minutes (except 1440 to 2160 minutes) after IV treatment, from 0 to 4320 minutes (except 30 and 1800 to 3600 minutes) after SC treatment, and from 0 to 4320 minutes (except 720 to 2160 minutes) after OTM treatment ($p < 0.05$) when compared with saline 0.9% (Table 1).

Table 1. Skin temperature (mean ± SD) in cats after saline (SAL) or buprenorphine administration via the three treatment routes: intravenous (IV), subcutaneous (SC), and buccal (OTM). † Significant difference from baseline values. * Significant difference from saline treatment (Two-way ANOVA).

		Time (hours)													
	Route	0	0.5	1	2	4	6	8	12	24	30	36	48	60	72
) C°(TS d bäraV	SAL (n = 6)	36.3 ± 0.4	36.4 ± 0.3	36.5 ± 0.1	36.2 ± 0.2	36.2 ± 0.2	36.3 ± 0.2	36.3 ± 0.3	36.4 ± 0.3	36.5 ± 0.2	36.5 ± 0.5	36.6 ± 0.4	36.4 ± 0.4	36.4 ± 0.3	36.4 ± 0.3
	IV (n = 6)	37.3 ± 0.5*	37.4 ± 0.8*	37.8 ± 0.5*	37.5 ± 0.7*	37.7 ± 0.5*	37.4 ± 0.3*	37.2 ± 0.2*	37.5 ± 0.6*	37.2 ± 0.4	36.9 ± 0.3	37.2 ± 0.3	37.1 ± 0.4*	37.1 ± 0.3*	36.8 ± 0.4
	SC (n = 6)	37.0 ± 0.3*	37.1 ± 0.6	37.7 ± 0.4†*	37.5 ± 0.5*	37.6 ± 0.8†*	37.6 ± 0.5*	37.6 ± 0.8†*	37.6 ± 0.4†*	37.3 ± 0.5*	37.1 ± 0.5	37.2 ± 0.6	37.0 ± 0.3	37.1 ± 0.2	37.1 ± 0.4*
	OTM (n = 6)	37.2 ± 0.4*	37.4 ± 0.5*	37.7 ± 0.3*	37.4 ± 0.3*	37.5 ± 0.4*	37.4 ± 0.4*	37.1 ± 0.3*	37.0 ± 0.5	37.2 ± 0.2	37.1 ± 0.3	37.1 ± 0.4	37.2 ± 0.3*	37.3 ± 0.5*	37.3 ± 0.4*

ST (Skin Temperature); SAL (Saline SC); IV (0.12 mg kg^{-1} buprenorphine IV);
SC (0.24 mg kg^{-1} buprenorphine SC); OTM (0.12 mg kg^{-1} buprenorphine buccal route of administration).

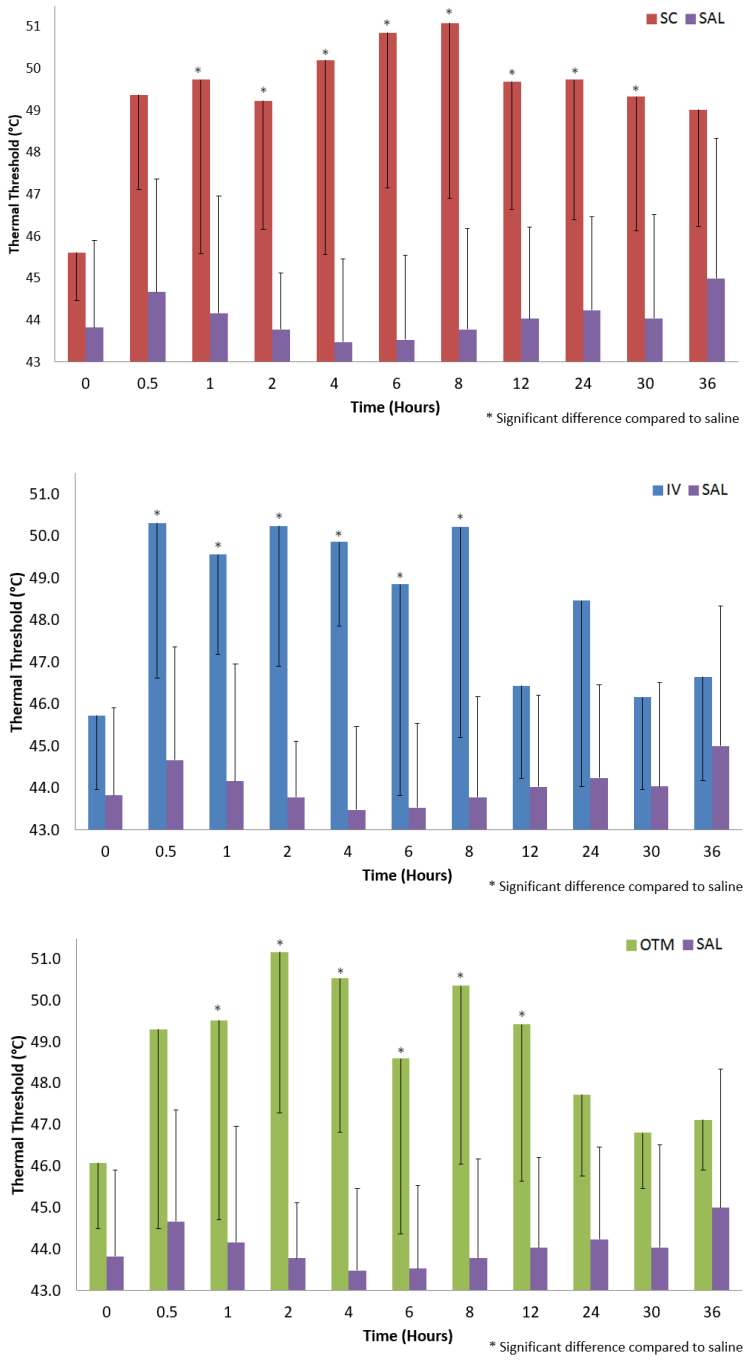
Mean ± SD baseline TT for all treatments (Phase I and II) was 45.3 ± 1.7°C. Thermal thresholds were not significantly different among IV, SC and OTM treatments. Thermal thresholds were significantly increased after IV treatment between 30 and 480 minutes (except 360 minutes; $p < 0.001$), after SC treatment between 60 and 1440 minutes (except 120 minutes; $p < 0.001$), and after OTM treatment between 60 and 480 minutes (except 360 minutes; $p < 0.001$) when compared with baseline values. Thermal thresholds were significantly increased after IV treatment from 30 to 480 minutes, after SC treatment from 60 to 1800 minutes, and after OTM from 60 to 720 minutes when compared with saline 0.9% ($p < 0.05$). Thermal thresholds did not increase after treatment with saline 0.9% (Table 2 and Figure 1).

Table 2. Thermal Threshold (mean ± SD) in cats after saline (SAL), or buprenorphine administration via the three treatment routes: intravenous (IV), subcutaneous (SC), and buccal (OTM). † Significant difference from baseline values. * Significant difference from saline (Two-way ANOVA).

		Time (hours)													
	Route	0	0.5	1	2	4	6	8	12	24	30	36	48	60	72
	SAL (n = 6)	43.8 ± 2.1	44.7 ± 2.7	44.2 ± 2.8	43.8 ± 1.3	43.5 ± 2.0	43.5 ± 2.0	43.8 ± 2.4	44.0 ± 2.2	44.2 ± 2.2	44.0 ± 2.5	45.0 ± 3.3	44.6 ± 1.8	43.8 ± 1.7	44.0 ± 1.8
	IV (n = 6)	45.7 ± 1.8	50.3 ± 3.7†*	49.6 ± 2.4†*	50.2 ± 3.3†*	49.9 ± 2.0†*	48.9 ± 5.0*	50.2 ± 5.0†*	46.4 ± 2.2	48.5 ± 4.4	46.2 ± 2.2	46.7 ± 2.5	45.1 ± 1.9	45.7 ± 2.4	46.7 ± 2.6
Variable TT (°C)	SC (n = 6)	45.6 ± 1.1	49.4 ± 2.3	49.7 ± 4.1†*	49.2 ± 3.1*	50.2 ± 4.6†*	50.9 ± 3.7†*	51.1 ± 4.2†*	49.7 ± 3.1†*	49.7 ± 3.3†*	49.3 ± 3.2*	49.0 ± 2.8	48.0 ± 4.0	45.1 ± 1.7	45.8 ± 1.6
	OTM (n = 6)	46.1 ± 1.6	49.3 ± 4.8	49.5 ± 4.8†*	51.2 ± 3.9†*	50.5 ± 3.7†*	48.6 ± 4.2*	50.4 ± 4.3†*	49.4 ± 3.8*	47.7 ± 2.2	46.8 ± 1.3	47.1 ± 1.2	46.0 ± 1.2	46.7 ± 1.4	46.4 ± 1.2

TT (Thermal Threshold); SAL (Saline SC); IV (0.12 mg kg^{-1} buprenorphine IV); SC (0.24 mg kg^{-1} buprenorphine SC); OTM (0.12 mg kg^{-1} buprenorphine buccal route of administration).

Figure 1. Thermal Threshold (mean ± SD) in cats after saline (SAL), or buprenorphine administration via the three treatment routes: intravenous (IV), subcutaneous (SC), and buccal (OTM).

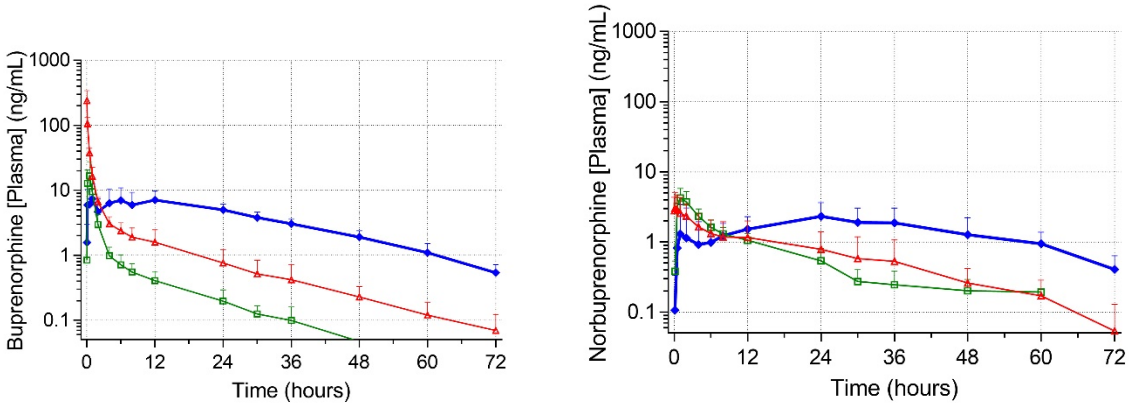


* Significant difference from saline (Two-way ANOVA). SAL (Saline SC); IV (0.12 mg kg^{-1} buprenorphine IV); SC (0.24 mg kg^{-1} buprenorphine SC); OTM (0.12 mg kg^{-1} buprenorphine buccal route of administration).

Blood sampling

A central jugular catheter was removed by one cat at 24 hours after IV administration of Simbadol™. Figure 2 shows mean plasma concentration profiles for buprenorphine and norbuprenorphine (Fig 2)

Figure 2. Mean plasma concentrations of buprenorphine and norbuprenorphine (± SD) in six conscious cats.

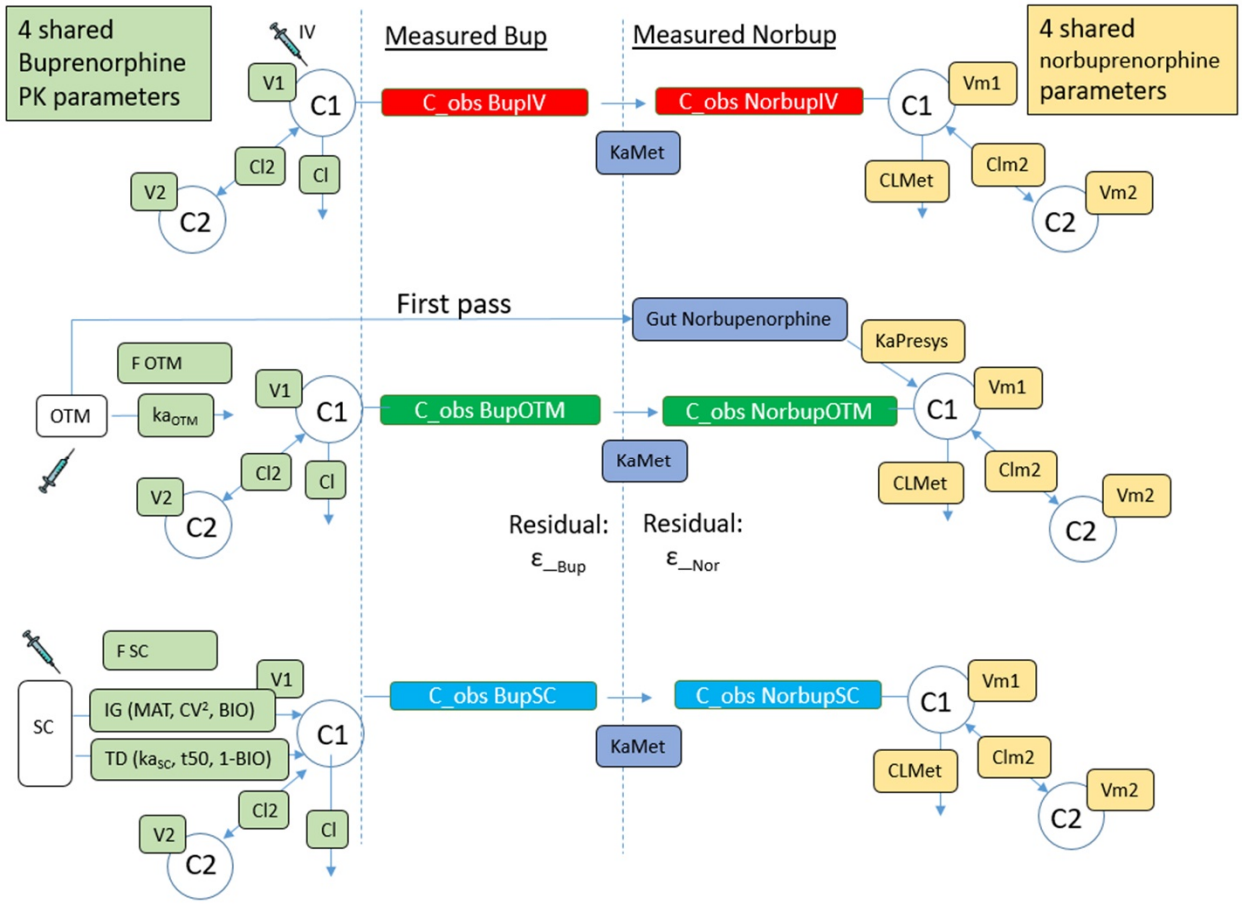


IV (0.12 mg/kg buprenorphine IV, red triangles); SC (0.24 mg/kg buprenorphine SC, blue diamonds); OTM (0.12 mg/kg buprenorphine buccal route of administration, green squares); [Plasma] (Plasma concentration).

Pharmacokinetic modeling and parameters

Figure 3 shows the structure of the final population PK model for buprenorphine and norbuprenorphine (Fig 3). For all parameters listed below, the inter-individual variability (IIV %) is reported immediately following each estimate where appropriate.

Figure 3. Pharmacokinetic-pharmacodynamic (PK-PD) model representation for buprenorphine and norbuprenorphine after subcutaneous, intravenous and buccal administration in six cats.



For the SC route, combined Inverse Gaussian (IG, rapid but short lasting) and Time-dependent (TD, delayed and progressive onset) inputs. Buprenorphine central PK parameters; clearance (CL), volume of distribution of the central compartment (V1), intercompartmental clearance (CL2), volume of distribution of peripheral compartment (V2). Norbuprenorphine central PK parameters: clearance (CLMet), volume of distribution of the central compartment (V1Met), intercompartmental clearance (CL2Met) and volume of distribution of the peripheral compartment (V2 Met). Rate constant of transformation from parent to metabolite (KaMet), first pass norbuprenorphine absorption rate (Kafirst-pass). PK parameters specific to OTM route: bioavailability (FOTM, parent and metabolite), absorption rate constant (kaOTM). PK parameters specific to SC route: bioavailability (FSC), proportion taken by IG input (BIO) and time-dependent delayed input (1-BIO), mean input rate time (MAT) was 7.21 h (3.5%) and variance of the input time (CV), maximal absorption rate constant (kaSC), time to achieve 50% of this maximum rate (T50).

For buprenorphine, a model that best predicted the individual observed plasma concentrations after SC route (see S3 - Appendix 3), and that has been previously used for systemic absorption of local anesthetic after perineural administration (16), was used. This model combined Inverse Gaussian (IG, rapid but short duration) and Time-dependent (TD, delayed and progressive onset) inputs. The three routes of administration shared four central PK parameters; clearance (CL = 0.98 L kg^{-1} $hour^{-1}$, 2.4%), volume of distribution of the central compartment (V1 = 0.75 L kg^{-1}, 11.3%), intercompartmental clearance (CL2 = 0.70 L kg^{-1} $hour^{-1}$, 0%) and peripheral volume of distribution (V2 = 7.15 L kg^{-1}, 8.1%) with a common proportional residual error term. The total body clearance of buprenorphine was moderate to high according to Toutain et al. 2004 (153). Volume of distribution at steady-state (Vdss) was 7.89 L kg^{-1}. The average beta elimination half-life for this bicompartmental model was 12.3 hours (154).

For PK parameters, specific to the OTM treatment, the bioavailability F_{OTM} was 23.6% (IIV 25%) and the absorption rate constant (ka_{OTM}) was 1.67 $hour^{-1}$ (25.1%), yielding an absorption half-life of 0.42 hours. For the SC treatment, the total bioavailability F_{SC} was 94% (IIV 23.4%). A proportion of 10.1% (IIV 113%) of F_{SC} was absorbed through early uptake (BIO = proportion taken by IG input) and the rest (1-BIO = 89.1%) was absorbed through a time-dependent delayed input (TD). For the IG input of the SC absorption, mean input rate time (MAT) was 7.21 hours (3.5%) and variance of the input time (CV) was 5.46 (0.5%). This translated into an initial peak of plasma concentration observed at 0.08 hours (5 minutes, at T_{max}, the mode of the IG function) corresponding to 10% of the buprenorphine being rapidly absorbed. For the TD input, the maximal absorption rate constant (ka_{SC}) was 0.062 $hour^{-1}$ (14.9%), yielding a slow late absorption half-life (11.2 hours). The time to achieve 50% of this maximum rate (T_{50}) was 2.8 hour (27%).

For norbuprenorphine, it was thought that it was exclusively generated from plasma buprenorphine degradation, with Ka_{Met} the irreversible conversion rate constant from the parent drug to its metabolite. The PK of norbuprenorphine was best described using a two compartment model for which four parameters common to the three administration routes could be estimated: norbuprenorphine clearance (CL_{Met} = 0.42 L kg^{-1} $hour^{-1}$, IIV 4.3%), volume of distribution of the central compartment ($V1_{Met}$ = 0.323 L kg^{-1}, 0.1%), intercompartmental clearance ($CL2_{Met}$ = 7.662 L kg^{-1} $hour^{-1}$) and volume of distribution of the peripheral compartment (and $V2_{Met}$ = 4.69 L kg^{-1}). Random effects for $CL2_{Met}$ and $V2_{Met}$ were not estimated. In a second step, a first pass effect was included in the model to account for the higher norbuprenorphine exposure after OTM administration when compared with other routes (Fig 2). The rate constant of transformation from parent to metabolite was estimated using the IV and SC datasets (Ka_{Met} = 0.196 $hour^{-1}$, 19%) yielding a transformation half-life 3.5 hours. The Ka_{Met} was then fixed to allow estimation of the first pass norbuprenorphine absorption rate constant using full 3-routes dataset ($Ka_{first\text{-}pass}$ 0.626 $hour^{-1}$, 6.9%, absorption half-life 1.1 hours) and F_{OTM} (parent and metabolite OTM bioavailability). The amount absorbed as norbuprenorphine via first pass after OTM administration contributed to approximately 1% out of the value of F_{OTM} (23.6%).

PK-PD modeling - Several models were evaluated (S3 - Appendix 3) and the best one was selected based on the Bayesian Information Criterion value and the identifiability of parameters. The model could reliably estimate the effect of buprenorphine but not that of norbuprenorphine. A hypothetical effect-compartment accounted for the delay in attaining maximal effect in relation to drug concentrations in the central compartment (155). The link between effect site buprenorphine concentration (C_e) and thermal antinociceptive effect (E) was modelled with an E_{max} function according to equation 1:

$$Antinociceptive\ effect\ (E) = T_0 + \frac{E_{max} \times {C_e}^n}{{EC_{50}}^n + {C_e}^n}$$

where T_0 is the estimated baseline thermal threshold (°C), E_{max} is the estimated maximal effect (°C), EC_{50} is the plasma concentration achieving 50% of E_{max} and n is the slope parameter of the concentration effect curve. The transfer rate constant Ke0 was 0.52 $hour^{-1}$ (IIV 0%). The estimated maximal effect E_{max} was 5.62°C (IIV 0%) over the baseline thermal threshold T_0 = 46.1 (IIV 0%). Buprenorphine EC_{50} (potency) was 2.13 ng mL^{-1} (IIV 447%) with a slope (n) of 1.54 (91%).

Discussion

Subcutaneous administration of the drug provided long-lasting thermal antinociception (≥ 24 hours). These effects were prolonged compared with the IV (8 hours) and OTM (≥ 8 hours) treatments. The combined modelling approach provided a robust model to capture the complex absorption of the SC treatment. Despite limitations in the thermal antinociceptive model such as right censored data (due to the safety cut-off) and individual variability, the final PK-PD model could describe and predict the prolonged analgesic effects of Simbadol™ after SC administration due to is biphasic rapid and slow absorption kinetics.

The duration of thermal antinociception was consistently longer in this study compared with traditional doses of buprenorphine, regardless of route of administration. In previous studies that tested thermal antinociception of buprenorphine in cats, antinociception was reported between 4 and 12 hours following 0.01 mg kg^{-1} IM buprenorphine (63). Further investigation, including IV and OTM administration at doses of 0.01-0.02 mg kg^{-1} found shorter durations of antinociception (between 0.5 to 6 hours) (62, 67, 68, 148, 149, 152). Additionally, it appeared that low dose buprenorphine administered SC was ineffective, with near undetectable plasma concentrations that did not provide thermal antinociception (152). Clinical guidelines would not recommend the SC route of administration for the management of pain using low doses of buprenorphine (72). In the current study, the increased dose of buprenorphine prolonged the duration of antinociceptive effect in all three routes of administration, however it is clear that the SC route of administration provided superior duration when compared with all others. A recent study compared thermal antinociceptive effects of buprenorphine using different doses (0.02-0.24 mg kg^{-1}) and found thermal antinociception of up to 30 hours when 0.12 mg kg^{-1} or more was administered (66). In the study herein, the SC administration produced consistent elevations in TT when compared with either baseline or placebo treatments.

Joint modeling provided robust estimates of the pharmacokinetic parameters of Simbadol™ for several reasons. Traditional methods used for pharmacokinetic modeling of buprenorphine in cats have studied each route of administration separately (62, 70, 152), instead of pooling data from crossover studies to strengthen estimation of parameters shared across routes of administration (CL, V1, CL2, V2). In the present study, six individuals were used in a typical cross-over design, yielding 18 related (six cats, three routes of administration) plasma concentration-time curves. Amalgamation of rich PK data from different routes in the same model using non-linear mixed effect modelling is encouraged to optimize data, and increase statistical power to address complex absorption or disposition kinetics (144). In the present study, the slow SC absorption kinetic could be unraveled due to the inclusion of IV data in the model (during exploratory deconvolution exercise or in the final model), which is a crucial requirement to study atypical drug absorption profiles (156). The slow SC absorption resulted in a delayed T_{max} compared with the OTM route. However, the beta elimination half-life of 12.3 hours was still longer than the half-life of the slowest SC absorption input (11.2 hours for TD input), therefore ruling out a flip-flop phenomenon.

The kinetic of norbuprenorphine followed closely the kinetic of the parent drug. Not only peak norbuprenorphine concentrations appeared much earlier after IV or OTM when compared with SC administration, but the plasma norbuprenorphine concentration-time curve also displayed double peaks corresponding to the one observed for buprenorphine. Hence, joint modelling of buprenorphine-norbuprenorphine further corroborated the slow biphasic SC buprenorphine uptake using a robust model.

The biphasic input model used has been reported in a previous study where a double peak phenomenon in the plasma concentration-time curve of ropivacaine after femoral blockade was observed in people (157). After a rapid short-lasting absorption from the perineural space, the

secondary slower input was accounted by either (i) partitioning of the drug in the surrounding tissues and subsequent mobilization with exercise-induced increase in perfusion or (ii) initial precipitation in surrounding tissues due to a low aqueous solubility and gradual re-dissolution, creating a concentration gradient that promotes systemic absorption (16). It is possible that a similar process occurs in the cat following SC administration of Simbadol™.

The pharmacokinetic parameters estimated in the current study are similar to those reported in the literature (Table 3) using buprenorphine in cats (10, 62, 70, 152). Some variability exists due to different bioavailability particularly in routes other than IV. For example, the elimination $t_{1/2}$ was longer in the current study, yet the clearance and VD_{SS} are similar across studies. One potential limitation in the present study is the variable doses between OTM/IV and SC (0.12 mg kg^{-1} vs 0.24 mg kg^{-1}). In a study from Taylor et al, increasing dosages of buprenorphine (0.02 to 0.24 mg kg^{-1} SC) revealed saturation kinetics (Table 4) (3). Similar clearances were observed, however the lack of IV administration in the latter study does not allow for an estimate of bioavailability, making VD_{SS} comparisons difficult. Indeed, the elimination half-life was shorter in the current study when compared with Taylor et al. This is likely a result of the variable formulations used in that study, which might alter drug absorption.

Table 3. Median pharmacokinetic estimates according to different dosage regimens and studies.

Parameter	Units	Current Study	Hedges et al. 2014		Steagall et al. 2013			Robertson et al. 2003	Taylor et al. 2001	
Dose	µg kg^{-1}	120-240			20				10	
Route		All routes	IV	OTM	IV	IM	SC	OTM	IV	IM
Clearance or CL/F	L kg^{-1} $hour^{-1}$	0.98	1.4	3.8*	0.5	0.8*	-	0.51*	1	1.4*
$V_{D\text{-Steady State}}$	L kg^{-1}	7.9	11.6	25.9	2.9	10.3	-	3.4	7.1	8.9
Elimination half-life	Hour	12.3	9.8	8.9	7	7.7	-	5.8	6.9	6.3

Values converted from published estimates to standardize units. Intravenous (IV), Buccal (B), Intramuscular (IM), Subcutaneous (SC).
**Variable bioavailability F%.*

Table 4. Median pharmacokinetic estimates using high doses of buprenorphine by different routes of administration in cats.

Parameter	Units	Current Study	Taylor et al. 2016			
Dose	µg kg^{-1}	120-240	20	60	120	240
Route		All routes	SC	SC	SC	SC
Clearance or CL/F	L kg^{-1} $hour^{-1}$	0.98	-	1.0	0.92	0.94
$V_{D\text{-Steady State}}$	L kg^{-1}	7.9	-	0.58*	0.59*	0.66*
Elimination Half-life	Hour	12.3	2.7	22.4	19.7	17.2

*Values converted from published estimates to standardize units, Subcutaneous (SC), *Vdbeta/F (L/mL)*

Current literature on OTM or sublingual administration of buprenorphine has suggested that jugular sampling may be inappropriate for PK studies using this drug because it overestimates bioavailability due to sampling a vessel which drains the site of administration (158). There are two studies in particular in which this overestimation has been documented. One study found an F of 116% and the other 139% in cats (62) and horses (159), respectively. When carotid arterial, jugular venous and saphenous sampling sites were simultaneously compared in cats, F was reported as 32%, 47% and 23% respectively (158). These factors were considered in the design of the current study. However, placing a catheter in the carotid artery three times during the study and for prolonged periods was found to be risky with a potential for blood sampling failure. The authors chose to place a central venous catheter to collect samples from a central site, rather than peripheral venous site. A pilot study showed that placing these catheters via the jugular site (versus medial saphenous) was both easy and repeatable, and the sampling port would require minimal restraint of the cats. As a further step to minimize overestimation, cats receiving OTM treatment would have the dose administered in the contralateral cheek relative to the jugular catheter. The reported bioavailability of the OTM treatment in the current study (F = 23.6%) is closer to that of the peripheral venous sample obtained in the previous study (158).

The SC route of administration provided prolonged analgesia due to its sustained plasma concentrations and the relationship between plasma, effect-side concentrations and time course of antinociception predicted by the sequential population PK/PD model. Despite consistent thermal antinociception based on averaged group data, the fit of the pharmacodynamic model was less satisfactory on an individual basis. Great individual variability in response to thermal stimulation was observed, however, similar findings have been reported (16). Treatments produced significant and variable behavioral and TT changes that could have affected the quality of the PK/PD model fit.

Other limitations in the present study were the inclusion of a safety threshold that produces artificially truncated data. A log likelihood approach to model right censored data as proposed by Sadiq et al. (160) was attempted but did not allow successful modelling with Phoenix NLME. In addition, PD modelling has not been described for norbuprenorphine in cats. In other species, the norbuprenorphine is between 50 and 200-fold less potent than buprenorphine with regards to its respiratory depressant or antinociceptive effects (161). Multiple models including both drug and metabolite were attempted to evaluate the contribution of norbuprenorphine to thermal antinociception (S3 - Appendix 3), however the PD parameters for norbuprenorphine were not identified.

Conclusion

Subcutaneous administration of Simbadol™ (Buprenorphine HCl, 1.8 mg ml^{-1}) provided long-lasting thermal antinociception (≥ 24 hours) in conscious cats. These effects are prolonged compared with the IV (8 hours) and OTM (≥ 8 hours) treatments. Joint pharmacokinetic-pharmacodynamic modelling showed prolonged plasma concentrations for the SC route. Despite the difficulties with pharmacodynamic modelling, the final model strongly supported the long acting analgesia provided by the drug. Advanced mathematical modelling of pooled data from different routes or parent-metabolite drug combinations and different studies allows leveraging of information to improve the understanding of complex pharmacokinetics.

Acknowledgments

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Competing interests

Dr. Paulo Steagall has received speaker honoraria and provided consultancy services to Zoetis. Dr. Beatriz Monteiro has provided consultancy services for Zoetis Canada. Dr. Ludovic Pelligand has received honoraria and provided consultancy services to Zoetis. Dr. Daniel Edge is an employee of Zoetis. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

Supporting Information

S1 File. Acclimatization of cats

S2 File. Analytical methods for buprenorphine and norbuprenorphine

S3 File. Population pharmacokinetic-pharmacodynamic modelling

Appendix 1 – Acclimatization of cats

Cats were moved daily (Monday to Friday) into the laboratory room and housed individually in the cages for the study. The threshold device (see section on Thermal threshold testing) was applied to the cats, but not tested, for one and a half hours. The cats were then allowed to rest for another half hour in the cage. During the second week of acclimatization (Monday-Friday), the response to thermal stimulation for each cat was studied every 30 minutes during a one-hour period (two readings) with the stimulus applied and tested. This was done to familiarize the observer (BM) with the cat's individual responses. During testing and acclimatization, cats were free-ranging and unrestrained at all times. Water, toys and a litter box were available at all times during testing and acclimatization. Food was offered between testing periods. Two weeks before acclimatization, cats were visited by one observer (BM/GD/PS) on a daily basis (Monday-Friday) for at least 15 minutes. By the time that the study began, the cats had lived in the accommodation for at least one month.

Appendix 2 – Analytical methods for buprenorphine and norbuprenorphine

Plasma samples were analyzed for buprenorphine and norbuprenorphine using HPLC-MS/MS (High Performance Liquid-chromatography tandem mass spectrometry). The drug and its metabolite were extracted using a liquid-liquid preparation technique. A total of 250 µL of standard solution (10.0 ng mL^{-1} $^{2}H_3$-norbuprenorphine and $^{2}H_4$-buprenorphine) was added to 100 µL of sample. This mixture was alkalinized using 50 µL of 5M ammonium hydroxide in a borosilicate sample tube. The sample was then vortexed for five seconds. Four millilitres of ethyl acetate were added and the sample mixed again via rotation for 20 minutes. All samples were then centrifuged at ~3500 *g* for 10 minutes and the organic layer transferred into a new sample tube. The samples were evaporated at 50°C under a stream of nitrogen. The dry residues were resuspended with 100 µL of 20:80 (v/v) methanol:water solution and transferred into injection vials for analysis. An isocratic mobile phase was used with a Phenomenex PFP(2) (150 x 3 mm I.D., 3 µm) and PFP (4 x 2.0 mm) security guard cartridge operating at 50°C. The mobile phase consisted of acetonitrile and 1.0% (v/v) formic acid in type 1 water at a ratio of 50:50, respectively. The flow rate was fixed at 0.30 mL min^{-1} and norbuprenorphine, buprenorphine and their respective internal standards ($^{2}H_3$-norbuprenorphine and $^{2}H_4$-buprenorphine) eluted at three and four minutes, respectively. Five µL of the extracted sample was injected and the total run time was set to five minutes. The mass spectrometer (MS) was interfaced with the high-performance liquid chromatography (HPLC) system using a pneumatic assisted heated electrospray ion source. MS detection was performed in positive ion mode, using selected reaction monitoring (SRM). Nitrogen was used for the sheath and auxiliary gases and was set at 50 and 15 arbitrary units. The HESI electrode was set to 3500 V. The capillary temperature was set at 350°C. Argon was used as collision gas at a pressure of 2.5 mTorr. Total cycle time was set at 0.25 seconds. Peak width of Q1 and Q3 were both set at 0.7 FWHM. Analysis of the sample assay method met standards for generally accepted bioanalytical chemistry, as such, sample analysis was performed (151). The result was the development of a HPLC-

MS/MS method which was validated for determination of feline plasma analysis for buprenorphine and norbuprenorphine.

Appendix 3: Population pharmacokinetic-pharmacodynamic modelling

A classic two compartment model with first order absorption was the starting point for modelling of the 3 routes simultaneously. It showed acceptable fitting for the IV and OTM routes, but failed to capture the multiple peaks displayed on the SC plasma concentration-time curve of all cats.

Preliminary deconvolution analysis:

To explore the complex absorption of buprenorphine SC, a deconvolution procedure was initially carried out to evaluate the shape of the SC input function over time (157). The PK disposition parameters from the IV fitting of the present study were used for this purpose. The biphasic input consisted of an initial sharp burst of absorption followed by a slower release phase. Therefore, a series of dual input models were tested to characterise the consistent double peak phenomenon observed after SC administration.

Goodness of fit:

For each Phoenix NMLE run, goodness of fit plots were prepared (162). The nested candidate models were compared on the basis of their biological plausibility, prediction based diagnostics (PRED, IPRED), residual-type diagnostics (RES and IRES), simulation based diagnostics (stratified VPC) and numerical diagnostics (minimisation of the Objective Function Value (OVF)). These were statistically tested with the Likelihood Test Ratio (when LRT was performed, deltaOVF >6.64; P<0.01, df = 1, otherwise the Akaike Information Criterion (AIC) was used) as along with measures of model stability and adequacy (convergence, precision of the parameters estimates).

Statistical description of the model:

Inter-animal variability was characterised with the assumption individual parameters were log-normally distributed around the population value (Eq. 1):

$$P_{ij} = \theta_j \times \exp(\eta_{ij}) \qquad (1)$$

Where P_{ij} is the *j*-th parameter value for individual *i*; θ_j is the typical value for the *j*-th parameter for the population; and η_{ij} is normally distributed around 0 with a variance of ω^2_j. To minimise the residual

variability (difference between predicted and observed values), additive and proportional error models were compared.

Parameters bound between 0 and 1 (typically bioavailabilities, noted F) were expressed and estimated in the model after a logit transform. The typical value of F (θ_F) was then used in equation 2 to yield a final estimate.

$$F_i = inv\ logit\ (\theta_F + \eta F_i) \quad (2)$$

Where F_i is the inverse logit of θF, the typical value of the bioavailability, and ηF_i is the residual for the i^{th} invidual.

The coefficient of variation of the PK parameters were approximated as follows (Eq. 3):

$$CV(\%) = \sqrt{exp\ (\omega^2) - 1}\ \times 100\% \quad (3)$$

Visual predictive checks were built to evaluate the performance of the final model by comparing the median of simulated (n=5000) plasma concentrations with observed data (+/- 5^{th} and 95^{th} percentiles).

PK modelling

Base model development for the SC administration

First, a 2 compartment model was composed to simultaneously fit the IV and the OTM. This allowed estimation of the physiological PK parameters common to the three routes of administration (namely CL, total body clearance; V, volume of the central compartment; CL2, intercompartmental clearance and V2, volume of the peripheral compartment). The model also provided the OTM absorption rate constant (ka_{OTM}) and absolute OTM bioavailability (F_{OTM}). The typical value θ_j and individual η_{ij} were fixed, thus reducing the number of parameters estimated in the complex modelling of SC absorption.

Zhou (156) reviewed strategies to model atypical absorption profiles. Several of these models were tested in combination to capture the double peak phenomenon (table 1). The best model was the from Gaudreault et al. (157) which combined an Inverse Gaussian (IG) input function and a time dependent (TD) input function.

Table1: Comparison of rival models for SC input function in joint IV, OTM and SC buprenorphine model and selection of best model

Combination for SC input function	OFV (-2LL)	AIC	Comment
Single FO Absorption	646	680	No capture of the second peak
Combination of two FO absorptions	626	644	Double peak not captured
Combination of two FO absorptions with tlag	599	621	Can capture double peak but misfit
Combination of two ZO absorptions with tlag	630	652	Double peak not captured
IG combined with FO absorptions with tlag	512	538	Good peak captures but FO restricts flexibility in late phase
Combination of two IG	644	670	Good initial peak capture but constant bias in late phase (prediction under-estimates concentrations)
IG combined with TD (gamma fixed)	498	526	Best fitting but identifiability issues and some peak misfits
IG combined with TD (gamma = CL)	***505***	***531***	***Best model: Excellent peak capture, predictions slightly under-estimate concentrations***

FO: first order, ZO: zero order, IG: inverse Gaussian input function, TD: time dependent input function.

First, a combination of two first order absorption phases were evaluated together to account for the final SC bioavailability (F_{SC}). The parameter BIO (value bound between 0 and 1) defined the proportion of the dose mobilised by the first process and consequently the proportion of the dose mobilised by the second process (1-BIO).

First order input was modelled as in equation 4:

$$I(t) = Dose \times F \times exp\,(-k_a \times t) \quad (4)$$

Second, an Inverse Gaussian distribution was evaluated to model the early and sharp peak of the buprenorphine input function (163). The density function of the Inverse Gaussian input can be written as a time-dependent function according to the following equation (Eq. 5):

$$I(t) = F_1 \cdot \left[\frac{MAT}{2\pi \times CV^2 \times t^3}\right]^{1/2} \cdot exp\left[-\frac{(t - MAT)^2}{2 \times CV^2 \times MAT \times t}\right] \quad (5)$$

With the 3 following parameters estimated: mean input rate time (MAT, representing the mean of the distribution), the variance of the input time distribution (CV^2, representing the skewness of the distribution) and the proportion of the SC bioavailability absorbed by this early process.

The mode of the IG distribution (t_{max}) is the time at which the input rate reaches its maximum. This was calculated as a more informative secondary parameter in order to facilitate interpretation of the values of MAT and CV^2 (163) (Eq 6):

$$t_{max} = MAT \times \left[\sqrt{1 + \frac{9}{4} \times CV^4} - \frac{3}{2} \times CV^2\right] \quad (6)$$

Third, Inverse Gaussian with time dependent absorption as in equation 7:

$$I(t) = \frac{F \cdot Ka}{[1+e^{-\left(\frac{t-t50}{\gamma}\right)}]} \quad (7)$$

Where k_a is the classic first-order absorption rate constant; t_{50} is the time required to achieve 50% of the maximal input rate; and γ is a shape parameter for the time-dependent process. Gamma (γ) was approximated by the value of clearance, as performed by Gaudreault et al.(157),

Pharmacodynamic modelling:

The full PK model (metabolite and parent drug) served as base for the sequential PKPD modelling. The different pharmacodynamic rival models, with or without the effect of the metabolite (norbuprenorphine), were compared (Table 2).

Of the four best models, none fit the data exceptionally well. The most complicated included 2 to 4 additional parameters which provided a slightly better fit, but were less identifiable than the most parsimonious model. The analgesic effect of norbuprenorphine in cats is unknown, this absence likely impacted the final PD model.

Table 2: final comparison of the 4 best PD models.

Pharmacodynamic model comparison (all with log normally distributed parameters and additive error model)	OFV (-2LL)	AIC	BIC	#Para-meters	Comment
A) Buprenorphine only: Effect compartment (Ke0) and Sigmoid Emax	***1881.1***	***1907.1***	***1966.8***	***13***	***Best model***
B) Buprenorphine only: Same model as above but inclusion of 3 equilibrations constants (Ke0): 1 for each route	1872.1	1906.1	1984.3	17	3 Ke0 physiologically unrealistic
C) Buprenorphine and Norbuprenorphine: Effect compartment model with competitive agonism, sharing the same Emax and Ke0 but with constraints on relative potency (different EC50)	1877.5	1907.5	1976.4	15	Better fitting but unrealistic estimates
D) Buprenorphine and Norbuprenorphine: same model as above but allowing 2 different constants for effect compartment Ke0 and Ke0m (metabolite)	1863.3	1897.3	1975.5	17	Unstable model

-2(LL): objective function value, AIC: Akaike Information Criterion, BIC: Bayesian Information Criterion

Ultimately the most parsimonious model (A) was adopted based on the BIC and the conservative identifiability. The absence of PK/PD data after administration of norbuprenorphine only precludes estimation of norbuprenorphine PD parameters in a joint model when taking into account the effect combined antinociceptive effect of parent drug and metabolite.

Model A is an effect compartment model with sigmoid E_{max} expression. In this model, a hypothetical pharmacodynamic compartment accounts for the delay in attaining maximal effect in relation to drug concentrations in the central compartment (155). The assumptions behind the effect-compartment model were: (i) first order processes (k_{e0} transfer rate constant for buprenorphine,) govern the onset and offset of the pharmacodynamics effect; (ii) the amount of drug in the effect compartment is negligible and does not affect plasma concentration, and (iii) the concentration in the effect compartment (C_e) and plasma are equal at steady state.

The link between effect site buprenorphine concentration (C_e) and plasma buprenorphine concentration was modelled with a differential equation (8):

$$\frac{dC_e}{dt} = ke_0 \times (C - C_e) \quad (8)$$

Where Ke0 is the equilibration rate constant between the central and the effect compartment.

The thermal antinociceptive effect (E) was modelled with an E_{max} function according to equation 9:

$$Antinociceptive\ effect\ (E) = T_0 + \frac{E_{max} \times {C_e}^n}{{EC_{50}}^n + {C_e}^n} \quad (9)$$

Where T_0 is the estimated baseline thermal threshold (°C); E_{max} is the estimated maximal effect (°C); EC_{50} is the plasma concentration achieving 50% of E_{max}; and n is the slope parameter of the concentration-effect curve.

LL models for right censored data

Due to safety cut-off (55 °C), the true TT for a number of timepoints would have been above this artificial limit to the nociceptive test (right censored data). Attempts to fit models which take into account right censored data were not successful (using log likelihood approach) This is a common problem in antinociception studies (160) and the implementation of this modelling approach is much easier in Monolix with the SAEM algorithm.

Results and goodness of fit plots:

The goodness of fit figures for the final PK model fitting (buprenorphine and metabolite) are included thereafter:

- Fig suppl. 1: observed values vs population prediction,
- Fig suppl. 2: observed values vs individual predictions,
- Fig suppl. 3: conditional weighted residuals vs time after dose,
- Fig suppl. 4: conditional weighted residuals vs population prediction,

- Fig suppl. 5 a. to f.: visual predictive check for buprenorphine IV (5.a), norbuprenorphine IV (5.b), buprenorphine OTM (5.c), norbuprenorphine OTM (5.d), buprenorphine SC (5.e), norbuprenorphine SC (5.f)

The goodness of fit figures for the final PD model fitting (buprenorphine only) are included thereafter:

- Fig suppl. 6: observed values vs population prediction,
- Fig suppl. 7: observed values vs individual predictions,
- Fig suppl. 8: conditional weighted residuals vs time after dose,
- Fig suppl. 9: conditional weighted residuals vs population prediction,
- Fig suppl. 10 a. to c.: pharmacodynamic visual predictive check for buprenorphine IV (10.a), OTM (5.b), buprenorphine SC (5.c)

The average trend (50^{th} percentile) of the data was well captured for the IV and SC initially. None of the models really captured the fast onset and intensity of OTM and all models tended to estimate the duration of analgesia beyond its real duration with SC.

Fig suppl. 1 (observed values vs population predictions)

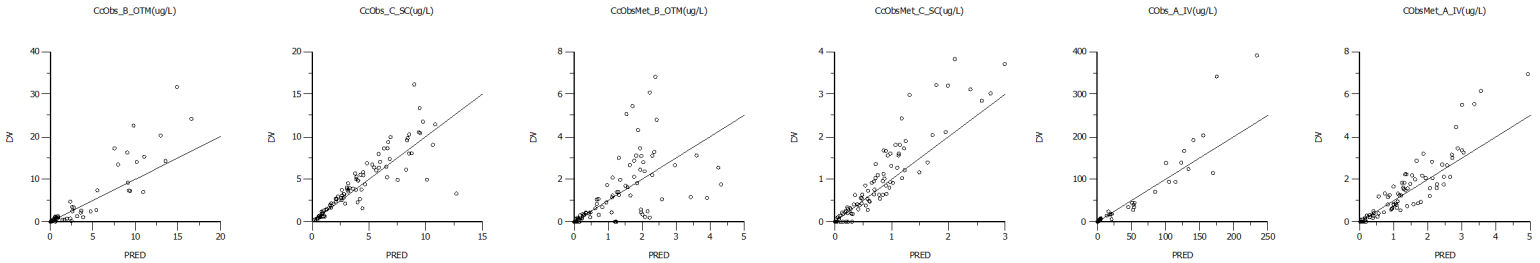
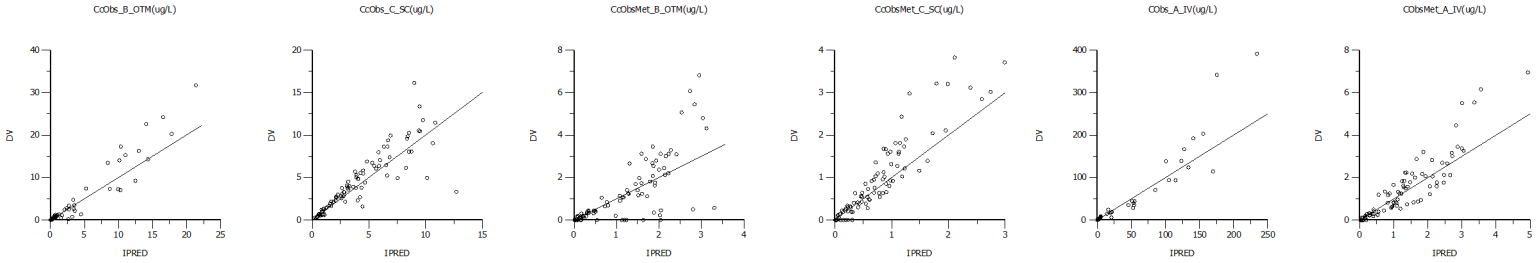


Fig suppl. 2 (observed values vs individual predictions)



Legend: CObs_A_IV: buprenorphine after IV administration, CObsMet_A_IV: norbuprenorphine after IV administration, CcObs_B_OTM: buprenorphine after OTM administration, CcObsMet_B_OTM: norbuprenorphine after OTM administration, CcObs_C_SC: buprenorphine after SC administration, CcObsMet_C_SC: norbuprenorphine after SC administration, DV = dependent variable (observed value), PRED = population predictions, IPRED = individual predictions

Fig suppl. 3 (conditional weighted residuals vs time after dose)

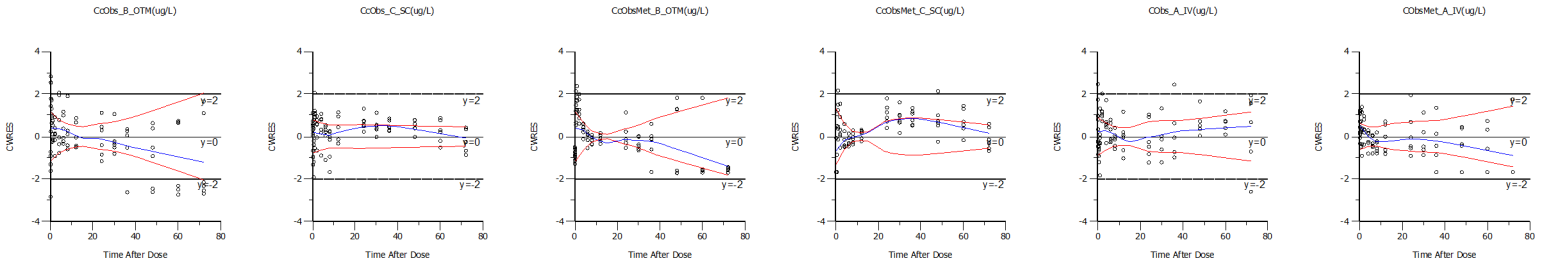
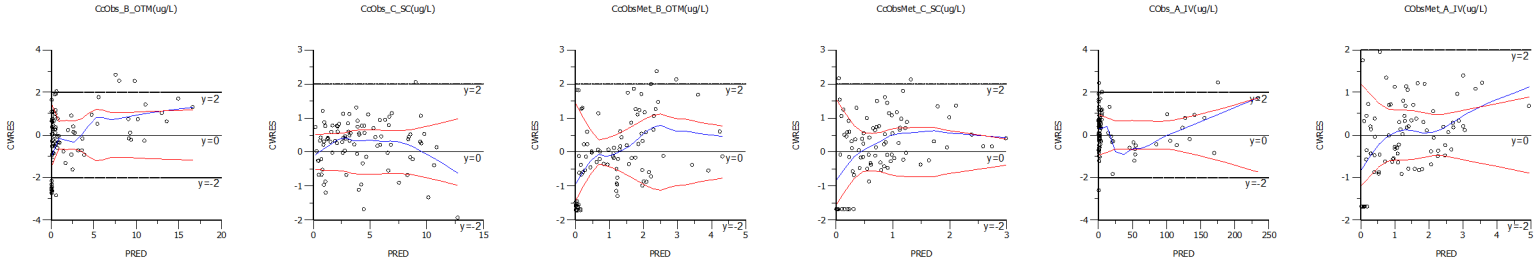


Fig suppl. 4 (conditional weighted residuals vs population prediction)



Legend: CObs_A_IV: buprenorphine after IV administration, CObsMet_A_IV: norbuprenorphine after IV administration, CcObs_B_OTM: buprenorphine after OTM administration, CcObsMet_B_OTM: norbuprenorphine after OTM administration, CcObs_C_SC: buprenorphine after SC administration, CcObsMet_C_SC: norbuprenorphine after SC administration, CWRES = conditional weighted residual, PRED = population predictions

Fig suppl. 5a: Stratified Visual Predictive Check (buprenorphine IV PK)

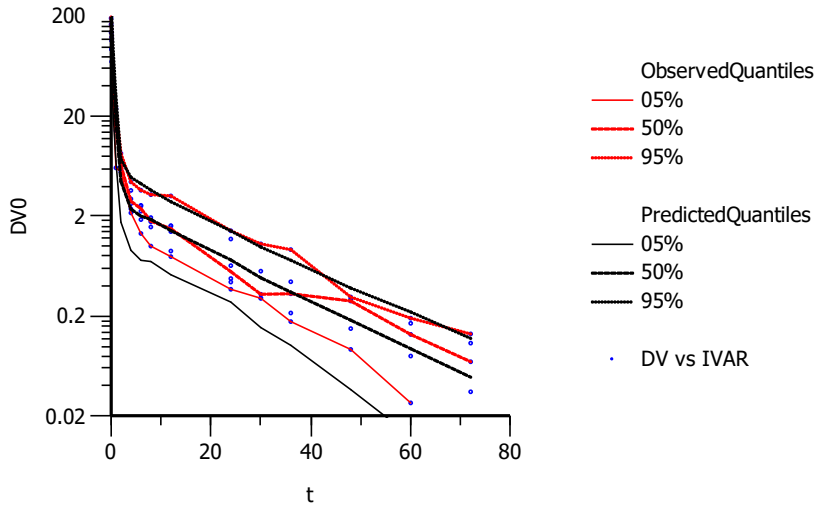


Fig suppl. 5b: Stratified Visual Predictive Check (norbuprenorphine IV PK)

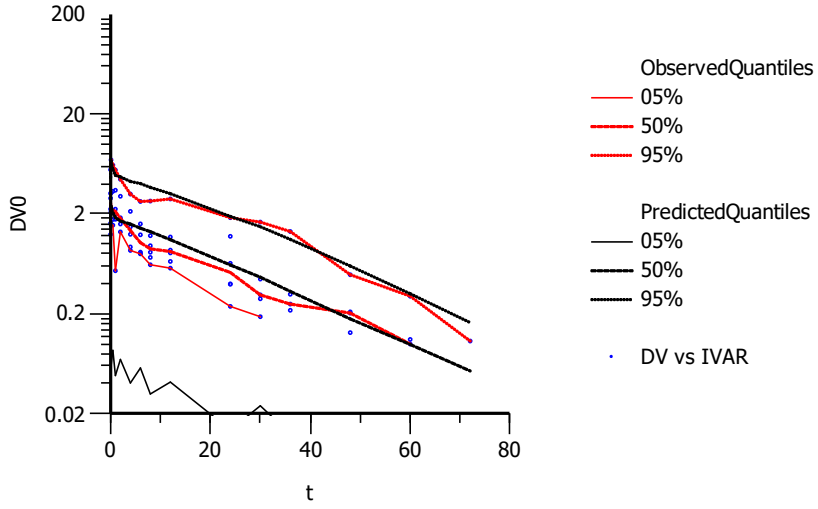


Fig suppl. 5c: Stratified Visual Predictive Check (buprenorphine OTM PK)

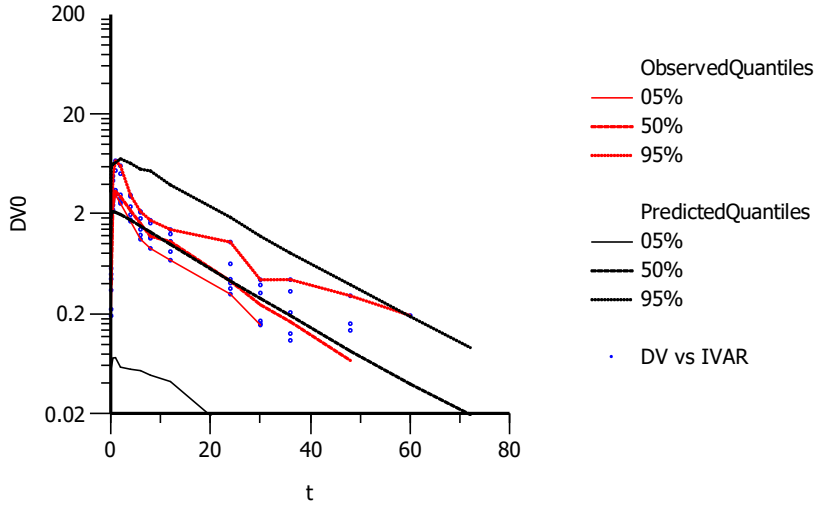


Fig suppl. 5d: Stratified Visual Predictive Check (norbuprenorphine OTM PK)

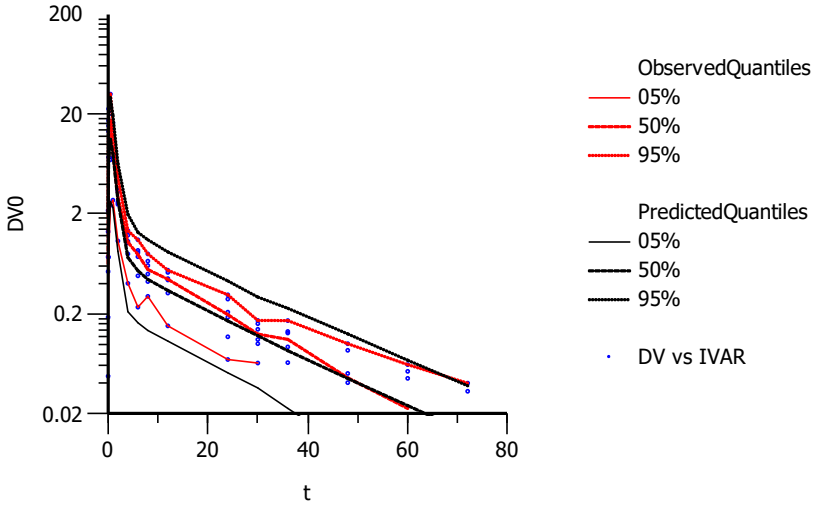


Fig suppl. 5e: Stratified Visual Predictive Check (buprenorphine SC PK)

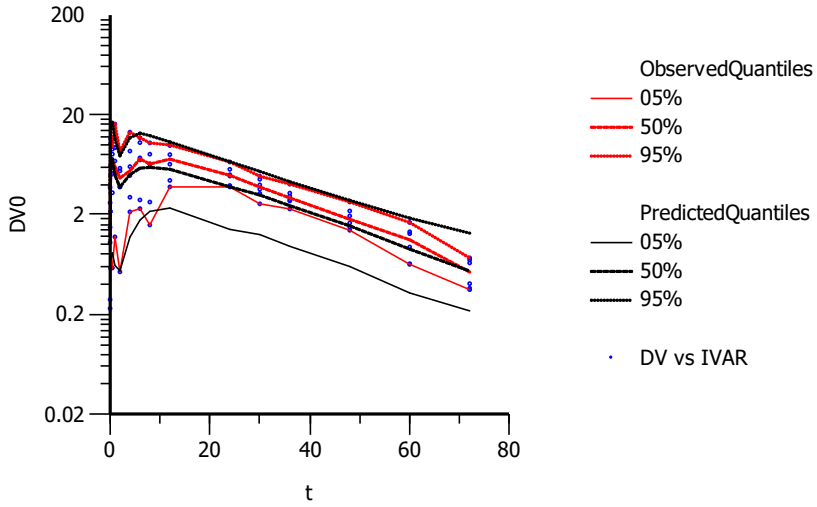
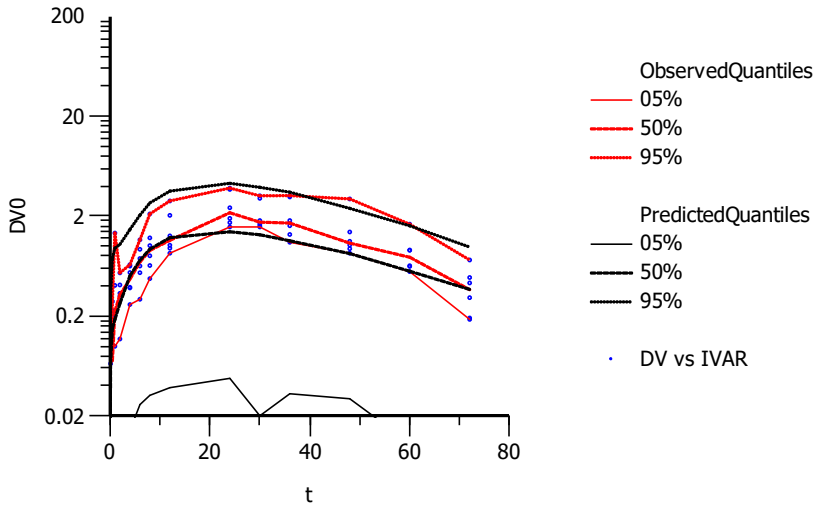
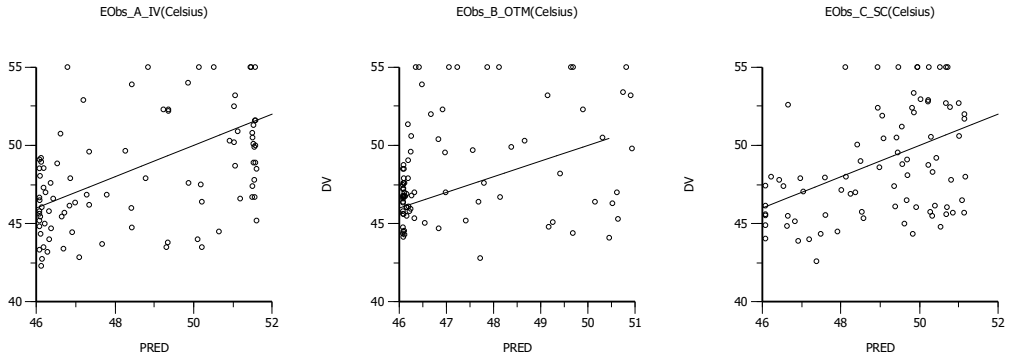


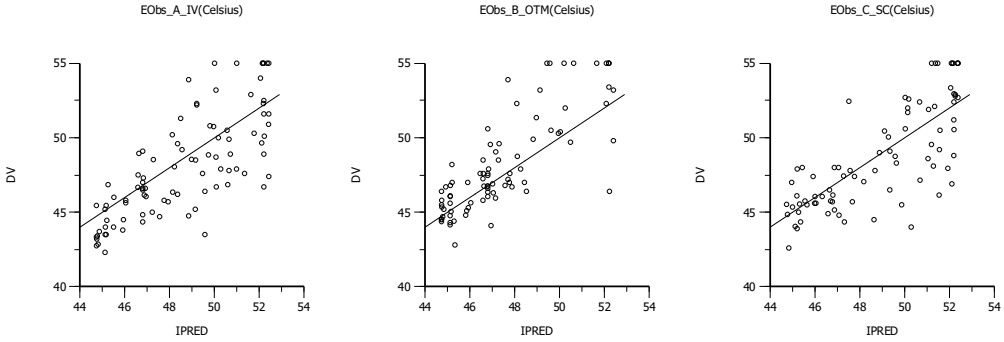
Fig suppl. 5f: Stratified Visual Predictive Check (norbuprenorphine SC PK)



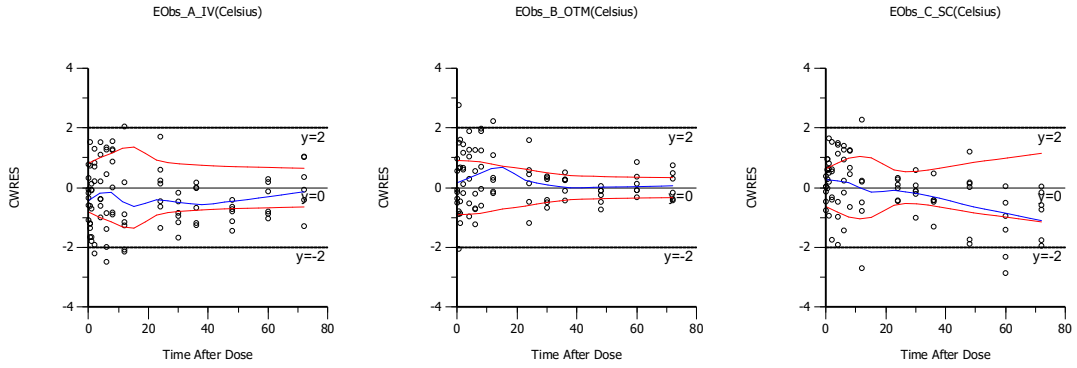
- Fig suppl. 6: observed PD values vs population prediction,



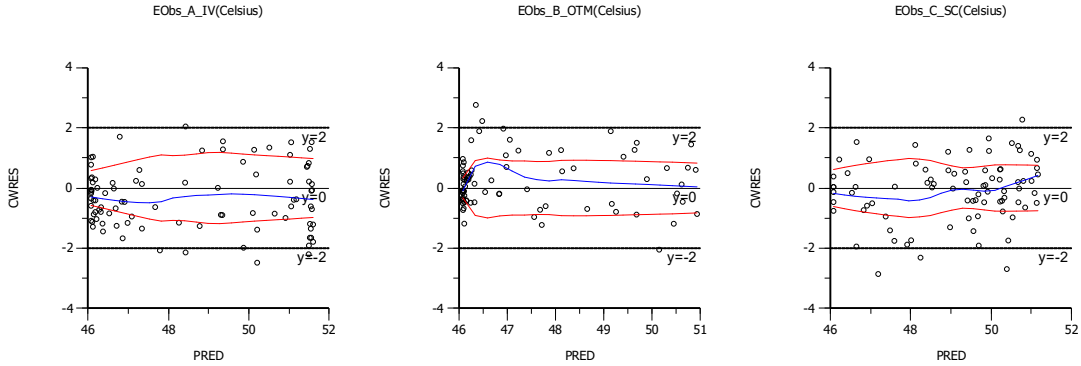
- Fig suppl. 7: observed PD values vs individual predictions,



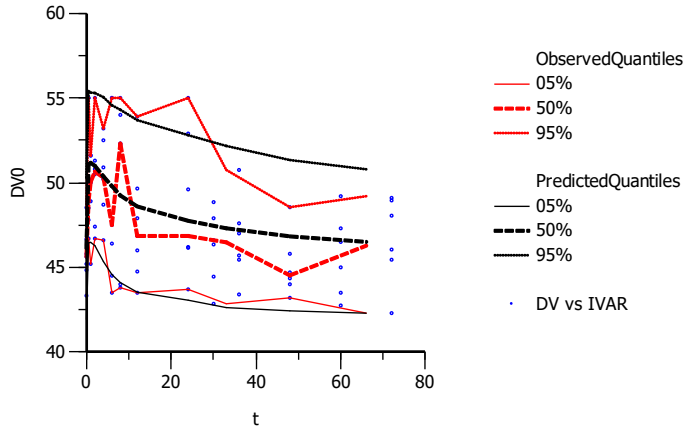
- Fig suppl. 8: conditional weighted residuals on PD vs time after dose,



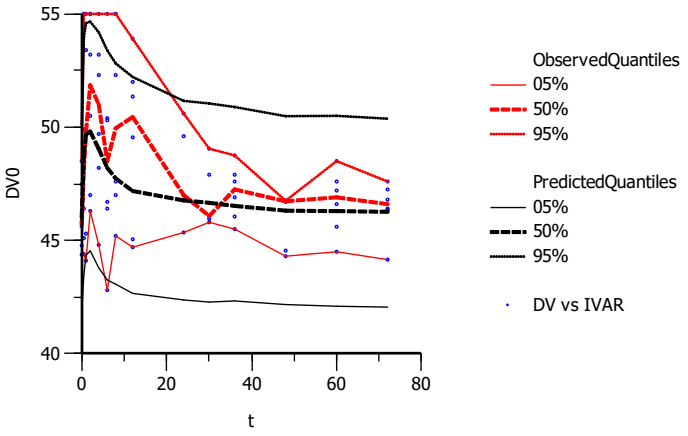
- Fig suppl. 9: conditional weighted residuals on PD vs population prediction,



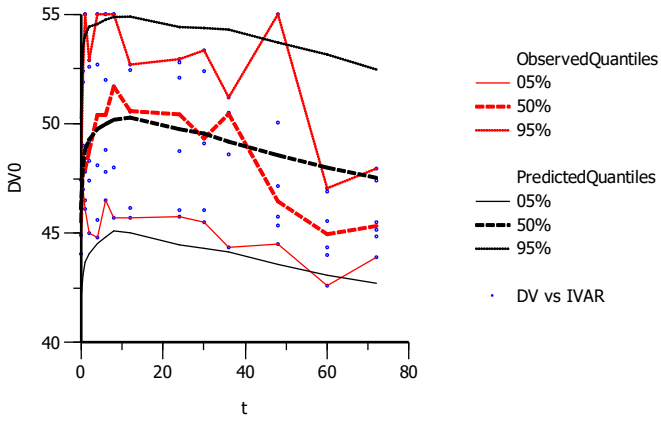
- Fig suppl 10.a: Stratified VPC: Pharmacodynamics fitting IV (DV0 = dependent variable = thermal threshold)



- Fig suppl 10.b: Stratified VPC: Pharmacodynamics fitting OTM



- Fig suppl 10.c: Stratified VPC: Pharmacodynamics fitting SC



Article 2

Thermal antinociception following oral administration of tapentadol in conscious cats

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This paper has been reproduced with the explicit consent of the co-authors.

Authors' contributions

GMD: project design, ethics preparation, thermal threshold evaluation, data acquisition, statistical analysis, preparation of manuscript. MCE: project preparation, treatment administration, data acquisition, data management, manuscript revision. PVMS: project design, funding, statistical analysis, manuscript revision.

Abstract

Objective To evaluate the onset, magnitude and duration of thermal antinociception after oral administration of two doses of tapentadol in cats.

Study design Prospective, randomized, blinded, experimental study.

Animals Six healthy adult cats weighing 4.4 ± 0.4 kg.

Methods Skin temperature (ST) and thermal threshold (TT) were evaluated using a wireless TT device up to 12 hours after treatment. Treatments included placebo (PBO, 50 mg dextrose anhydrase orally), buprenorphine (BUP, 0.02 mg kg^{-1}) administered intramuscularly, low-dose tapentadol (LowTAP, 25 mg orally; mean 5.7 mg kg^{-1}) and high-dose tapentadol (HighTAP, 50 mg orally; mean 11.4 mg kg^{-1}) in a blinded crossover design with 7 day intervals. Statistical analysis was performed using ANOVA with appropriate *post hoc* test ($p \leq 0.05$).

Results Salivation was observed immediately following 11 out of 12 treatments with tapentadol. The ST was significantly increased at various time points in the opioid treatments. Hyperthermia (≥ 39.5 °C) was not observed. Baseline TT was 45.4 ± 1.4°C for all treatments. Maximum TT values were 48.8 ± 4.8 °C at 1 hour in LowTAP, 48.5 ± 3.0 °C at 2 hours in HighTAP and 50.2 ± 5.3 °C at 1 hour in BUP. TT significantly increased after LowTAP at 1 hour, after HighTAP at 1–2 hours, and after BUP at 1–2 hours compared with baseline values. TTs were significantly increased in BUP at 1–2 hours compared with PBO.

Conclusion and clinical relevance Oral administration of tapentadol increased ST and TT in cats. The durations of thermal antinociception were similar between HighTAP and BUP, both

of which were twice as long as that in LowTAP. Studies of different formulations may be necessary before tapentadol can be accepted into feline practice.

Keywords analgesia, feline, opioid, pain, tapentadol.

Introduction

Tapentadol is a novel atypical opioid drug with a dual mechanism of action. The drug is an agonist of mu-opioid receptors and inhibits nor-epinephrine re-uptake (104). Unlike tramadol, tapentadol is administered in the active form and does not require hepatic metabolism for an analgesic effect. The efficacy of tapentadol is similar to that of morphine in the treatment of acute and chronic pain in humans (108). Tapentadol does not induce or inhibit hepatic cytochrome P450 enzymes (106). This is considered to be an advantage, as it can be administered to patients with mild to moderate renal or hepatic impairment (107).

In veterinary medicine, studies have been published on the pharmacokinetics of tapentadol in different species (109-111). In dogs and cats, the pharmacokinetics of tapentadol after parenteral administration have been described (112, 113); however, clinical application of these results is limited, as the drug is only commercially available in tablet form (107). Bioavailability (F) of tapentadol is low after oral administration at 4.4%, 9% and 32% in dogs, rats and humans, respectively (112, 114).

Tapentadol is metabolized in humans by hepatic glucuronidation by uridine 5'-diphospho-glucuronosyl transferases (UGTs) UGT1A9 and UGT2B7 (105, 106). The low capacity of glucuronidation is a well-known therapeutic issue in cats mitigated by longer dosing intervals and reduced dosages of therapeutics metabolized by this route. Cats have the ability to perform phase II glucuronidation, but a lack of specific isoenzymes limits their ability to metabolize certain drugs (e.g. acetaminophen). The UGT1A9 isoenzyme is absent in the cat and the production of UGT2B7 is limited (164).

Low bioavailability after oral administration of tapentadol in other species is a direct result of normal hepatic glucuronidation. Therefore, cats may not metabolize tapentadol in the same manner, and bioavailability may be increased, providing analgesic effects (112). In one study, longer durations of plasma concentrations of tapentadol were measured in cats after intravenous (IV), intramuscular (IM) and subcutaneous administration than were seen in other species (113). Agitation, panting and salivation were the main adverse effects after IV administration and are similar to those in other species (113). These differences in metabolism of tapentadol between cats and other species, in addition to the lack of pharmacodynamic studies, suggest that there is a need for a study assessing the analgesic (antinociceptive) properties and adverse effects of tapentadol in cats.

The aim of this study was to evaluate the onset, magnitude and duration of thermal antinociception after oral administration of two doses of tapentadol in cats. We hypothesized that tapentadol would increase thermal thresholds (TTs) with greater effect at a higher dose in awake cats.

Materials and methods

The study protocol was approved by the animal care committee of the University of Montreal (protocol no. 15-Rech-1780). This study is reported according to the 'Animal Research: Reporting of *In Vivo* Experiments' (ARRIVE) guidelines (145).

Animals, study design and treatments

Six healthy adult cats, two females and four males, weighing 4.4 ± 0.4 kg [mean ± standard deviation (SD)] were studied in a randomized, prospective, controlled, blinded and crossover study with ≥ 7 days between experiments. Cats were housed according to the Canadian Animal Care Council guidelines. The cats were fed a commercially available diet twice daily with *ad libitum* water. Environmental enrichment was provided following American Association of Feline Practitioners and International Society of Feline Medicine guidelines (146). During testing, cats were housed individually in adjacent cages (67 × 55 × 68 cm) in a room with light, temperature and humidity control. Cats were acclimated to the testing procedures several weeks before the study began.

The commercial tablet form of tapentadol in its immediate-release formulation (Nucynta IR 50 mg tablet; Janssen, Inc., ON, Canada) was used for this study. Four treatments for each cat were randomized using online software (www.randomization.org): placebo (50 mg dextrose anhydrase capsule, treatment PBO) administered orally; buprenorphine (0.02 mg kg^{-1}; Vetergesic, 0.3 mg mL^{-1}; Champion Alstoe, ON, Canada; treatment BUP) IM; low-dose tapentadol (mean = 5.7 mg kg^{-1}; one-half of a 50 mg tablet; treatment LowTAP) orally; and high-dose tapentadol (mean = 11.4 mg kg^{-1}; 50 mg tablet; treatment HighTAP) orally. Oral administration of tapentadol and placebo was performed using a commercial piller device (Pet-Piller; Jorgensen Laboratories, Inc., CO, USA). Water (3 mL) was administered to the cats after oral administration of treatments using a syringe. All treatments were administered by individuals who were not involved with TT testing.

Thermal thresholds

Antinociception was evaluated using a wireless thermal threshold device (WTT1; Topcat Metrology Ltd, UK). The device is incorporated into an elasticated vest, containing a wireless receiver, power supply and thermal probe. The device records skin temperature (ST). Afterwards, the evaluator triggers a ramped thermal stimulus (0.6 °C second^{-1}) and it is stopped when the cat exhibits a nociceptive behavioral response (eg. vocalization, rolling, jumping) which is considered the TT, or when the cut-off temperature of 55 °C is reached. The device was calibrated and maintained according to the manufacturer's recommendations. TT testing was evaluated before (baseline) and at 0.5, 1, 2, 3, 4, 6, 8, and 12 hours after treatment administration. Baseline values were determined after an acclimation period of 30 minutes using the mean of three recordings performed at 15 minute intervals prior to treatment. A single evaluator (GMD), blinded to the treatment order, performed the TT testing.

Adverse effects and behavioral changes

Any behavioral changes, adverse reactions or additional observations were recorded during the testing period. A five-point simple descriptive scale (0–4, where 0 is baseline normal behavior) was used to score behaviors observed during the testing period (Appendix 1). Behavior scores were recorded at each time point.

Statistical analysis

Prior to the testing phase, a prospective power analysis concluded that a sample size of six cats would be sufficient to detect mean temperature differences of > 3.2 °C with a power of 0.8 and an alpha level set at 0.05 based on the results of similar studies (65). Statistical analysis

was performed using GraphPad Prism, Version 5.0 (GraphPad Software, Inc., CA, USA). Temporal changes were analyzed using one-way ANOVA for repeated measures followed by Dunnett's test when appropriate. Treatment comparisons were made using two-way ANOVA followed by Bonferroni's correction ($p \leq 0.05$). ST and TT values (°C) were used for comparisons.

Results

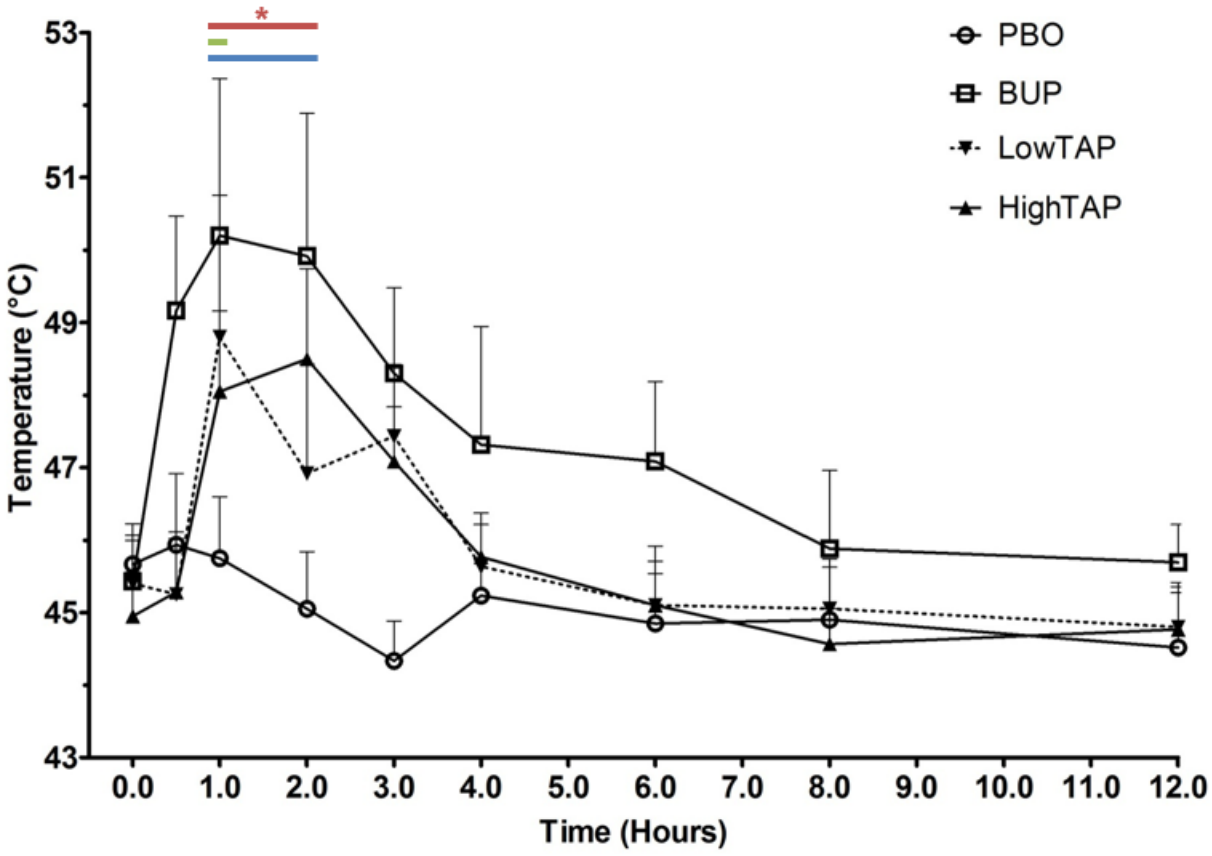
There were no adverse effects recorded in PBO or BUP. Excessive salivation lasting < 20 minutes was observed in five of six cats in LowTAP and six of six cats in HighTAP immediately following treatment administration. Table 1 shows the behavior scores during the study. Dysphoria (score 4) was not observed..

Baseline ST for all treatments (n = 24) was 36.3 ± 0.3 °C. ST was not significantly increased after PBO. ST was significantly increased at 0.5–2 hours in LowTAP (p = 0.0007), at 0.5–8 hours (p = 0.0001) in HighTAP (except at 4 hours), and at 1 hour in BUP (p = 0.02) as compared with baseline (Table 2). Hyperthermia (≥ 39.5 °C) was not recorded. Significant differences were not detected among treatments. The peak values for each treatment were 36.7 ± 0.3 °C at 1 hour in PBO, 37.0 ± 0.6 °C at 1 hour in BUP, 37.0 ± 0.3 °C at 0.5 hours in LowTAP, and 37.2 ± 0.4 °C at 0.5 hours in HighTAP.

Baseline TT for all treatments (n = 24) was 45.4 ± 1.4 °C (Table 3). TTs did not increase significantly after PBO. They were significantly increased at 1–2 hours in BUP (p = 0.02), at 1 hour in LowTAP (p = 0.05) and at 1–2 hours in HighTAP (p = 0.002) as compared with baseline. TTs were significantly increased in BUP at 1–2 hours (p = 0.05) as compared with PBO but there

were no significant differences between tapentadol treatments and placebo (Figure 1 & Table 3).

Figure 1. Thermal threshold (°C; mean ± standard error) in cats after administration of placebo (PBO, 50 mg dextrose orally), buprenorphine (BUP, 0.02 mg kg^{-1} intramuscularly), low-dose tapentadol (LowTAP, 25 mg orally) and high-dose tapentadol (HighTAP, 50 mg orally). Baseline values (BL) were collected before treatment administration at T0. There were six cats in each treatment



*Significantly different from PBO, BUP only ($p \leq 0.05$). *Coloured bar* (Red – BUP; Green – LowTAP; Blue – HighTAP) Significantly different from BL ($p \leq 0.05$).

Table 1 Behavior scores [median (range)] in cats after administration of placebo (PBO, 50 mg dextrose orally), buprenorphine (BUP, 0.02 mg kg^{-1} intramuscularly), low-dose tapentadol (LowTAP, 25 mg orally) and high-dose tapentadol (HighTAP, 50 mg orally). Baseline (BL) values were collected before treatment administration at T0. There were six cats in each treatment

	Time (hours)								
Treatment	**BL**	**0.5**	**1**	**2**	**3**	**4**	**6**	**8**	**12**
PBO	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
BUP	0 (0–0)	1.5 (0–2)	2 (0–2)	2 (0–3)	2.5 (0–3)	2.5 (0–3)	2.5 (0–3)	1 (0–3)	0 (0–0)
LowTAP	0 (0–0)	0 (0–2)	0.5 (0–1)	0.5 (0–2)	0 (0–2)	0 (0–2)	0 (0–0)	0 (0–0)	0 (0–0)
HighTAP	0 (0–0)	0 (0–1)	1 (0–2)	0.5 (0–2)	1 (0–2)	0 (0–2)	0 (0–0)	0 (0–0)	0 (0–0)

Table 2 Skin temperature (°C; mean ± standard deviation) in cats after administration of placebo (PBO, 50 mg dextrose orally), buprenorphine (BUP, 0.02 mg kg^{-1} intramuscularly), low-dose tapentadol (LowTAP, 25 mg orally) and high-dose tapentadol (HighTAP, 50 mg orally). Baseline values (BL) were collected before treatments were administered at T0. There were six cats in each treatment

	Time (hours)								
Treatment	**BL**	**0.5**	**1**	**2**	**3**	**4**	**6**	**8**	**12**
PBO	36.4 ± 0.3	36.7 ± 0.4	36.7 ± 0.3	36.5 ± 0.3	36.4 ± 0.3	36.7 ± 0.5	36.6 ± 0.5	36.5 ± 0.3	36.6 ± 0.4
BUP	36.4 ± 0.3	36.8 ± 0.8	37.0 ± 0.6*	36.7 ± 0.4	36.4 ± 0.4	36.7 ± 0.4	36.4 ± 0.3	36.7 ± 0.6	36.5 ± 0.5
LowTAP	36.2 ± 0.2	37.0 ± 0.3*	36.8 ± 0.3*	36.7 ± 0.3*	36.6 ± 0.4	36.4 ± 0.2	36.7 ± 0.3	36.3 ± 0.4	36.6 ± 0.4
HighTAP	36.2 ± 0.2	37.2 ± 0.4*	37.0 ± 0.6*	36.9 ± 0.4*	36.9 ± 0.3*	36.6 ± 0.3	36.8 ± 0.2*	36.7 ± 0.3*	36.5 ± 0.3

*Significantly different from BL (p <0.02).

Table 3 Thermal threshold (°C; mean ± standard deviation) in cats after administration of placebo (PBO, 50 mg dextrose orally), buprenorphine (BUP, 0.02 mg kg^{-1} intramuscularly), low-dose tapentadol (LowTAP, 25 mg orally) and high-dose tapentadol (HighTAP, 50 mg orally). Baseline values (BL) were collected before treatment administration at T0. There were six cats in each treatment

	Time (hours)								
Treatment	**BL**	**0.5**	**1**	**2**	**3**	**4**	**6**	**8**	**12**
PBO	45.7 ± 1.4	45.9 ± 2.4	45.8 ± 2.1	45.1 ± 1.9	44.3 ± 1.3	45.2 ± 1.2	44.9 ± 1.7	44.9 ± 2.1	44.5 ± 2.2
BUP	45.4 ± 1.5	49.2 ± 3.2	50.2 ± 5.3*†	49.9 ± 4.8*†	48.3 ± 2.9	47.3 ± 4.0	47.1 ± 2.7	45.9 ± 2.6	45.7 ± 1.3
LowTAP	45.4 ± 1.5	45.3 ± 2.1	48.8 ± 4.8*	46.9 ± 3.7	47.4 ± 2.4	45.6 ± 1.4	45.1 ± 2.0	45.1 ± 1.4	44.8 ± 1.2
HighTAP	45.0 ± 1.5	45.3 ± 1.8	48.1 ± 2.7*	48.5 ± 3.0*	47.1 ± 1.8	45.8 ± 1.5	45.1 ± 1.5	44.6 ± 1.2	44.8 ± 1.4

*Significantly different from BL ($p \leq 0.05$). †Significantly different from PBO ($p \leq 0.05$).

Discussion

The results indicate that oral administration of tapentadol evoked thermal antinociception, with the higher dose having a prolonged effect in cats. The LowTAP treatment increased thermal antinociception at 1 hour and the HighTAP increased it for up to 2 hours. Additionally, HighTAP had a similar duration of thermal antinociception to that of buprenorphine. However, tapentadol did not significantly increase TT when compared with PBO. Large standard deviations precluded the ability of ANOVA to detect significant differences among treatments when using six cats. In addition, power analysis may have overestimated the antinociceptive effects of tapentadol, which would explain why there was no difference between the PBO and tapentadol treatments.

The thermal antinociceptive device has been validated in cats (60) and has been used for the study of opioid antinociception in several reports (51, 62, 63, 65, 66, 165). It has been proposed that duration of thermal antinociception after opioid administration is well correlated with clinical analgesia of the same drugs in this species (62, 63, 166). Tapentadol has a dual mechanism of action, and some support the nor-epinephrine re-uptake inhibition pathway as primarily responsible for analgesia. Thus, it is possible that TT testing is not an ideal model for evaluating antinociceptive effects of drugs with a dual mechanism of action. However, tramadol has a similar mechanism of action to tapentadol, and oral administration of tramadol has produced thermal antinociception in a dose-dependent manner (61). These findings show that TT testing can be employed in studies using drugs with dual analgesic effect. Nonetheless, it is clear that clinical pain is complex and that more than one type of nociceptive

stimulus (i.e. mechanical and thermal) should be used to evaluate analgesia comprehensively (69).

The duration of thermal antinociception after IM administration of buprenorphine (0.01 mg kg^{-1}) in cats was reported to be between 4 and 12 hours (63). Other studies have measured shorter periods of antinociception (between 1 and 5 hours) after buprenorphine (0.01 and 0.02 mg kg^{-1}) (67, 68, 148-150). Consequently, a review of buprenorphine effects in cats highlighted the advisability of continued pain assessment after buprenorphine administration as a second dose of buprenorphine may be required after 4 hours (72). The present study is unable to determine if the duration of TT elevation in the BUP and tapentadol treatments will correlate with clinical effect. Therefore, a clinical trial is warranted to determine the analgesic effects of tapentadol in comparison with buprenorphine in cats.

Skin temperatures were significantly increased in all opioid treatments as compared with baseline, but not among treatments. Administration of some opioids, including meperidine and hydromorphone, is followed by a significant elevation in body temperature (49, 114). However, hyperthermia was not observed in this study and the small but significant elevations in ST after tapentadol administration were considered to be of no clinical relevance. Euphoria, rolling and increased activity are associated with opioid treatments and may have contributed to the increased temperature observed in the present study.

Tapentadol resulted in profuse salivation in 11 out of 12 cats immediately after oral administration of both doses (5.7 and 11.4 mg kg^{-1}). This adverse effect was also observed after IV administration of 5 mg kg^{-1} (113). The cause of salivation is unclear but could be associated with the immediate-release formulation of tapentadol. People have commented

that tapentadol is a bitter-tasting drug. Formulations for human use typically address this problem by the inclusion of sweeteners to mask the taste or by providing formulations for alternate routes of administration (167). Cats have taste receptors capable of detecting bitter flavors, but they lack receptors for sweet flavors (168). Consequently, formulations with sweeteners intended for people may not be of benefit for oral administration in cats. Further study using alternate formulations or preparations should be considered, as the formulation used in this study may prohibit clinical use of tapentadol in cats.

In this study, the chosen doses of tapentadol were based on the pharmacokinetics after parenteral administration of the drug (5 mg kg^{-1}) in cats (113). The decision to increase the dose to 11.4 mg kg^{-1} in HighTAP was based on three main factors: 1) the low F of tapentadol after oral administration in other species; 2) the idiosyncrasies of feline hepatic metabolism and its potential impact on F, as previously discussed; and 3) the practicality of using a commercial formulation of tapentadol (50 mg tablet) in the study. One of the main limitations of the present study is the lack of pharmacokinetic data after oral administration of tapentadol in cats. Poor absorption of tapentadol could explain its limited antinociceptive effect and a pharmacokinetic study could explain how increases in doses would affect pharmacokinetic variables. Additionally, the metabolism of tapentadol has not been studied in cats and it is not clear if the low capacity for glucuronidation could indeed increase its bioavailability and analgesic effects in this species.

The results of this study indicate that oral administration of both doses of tapentadol (5.7 and 11.4 mg kg^{-1}) increased thermal antinociception in cats. The 2 hour duration of effect of the higher dose was the same as for buprenorphine (0.02 mg kg^{-1}) and twice as long as the

low dose of tapentadol. Tapentadol did not significantly increase TT when compared with placebo, regardless of dose. Large variations were seen, and perhaps the antinociceptive effects of tapentadol were overestimated in the prospective power analysis. Poor palatability was identified with the formulation of tapentadol used in this study. Studies of different formulations may be necessary before tapentadol can be accepted into feline practice.

Appendix 1 Behavior scoring scale

Score	Description
0	*Normal* cat behavior. Similar to before drug administration.
1	Signs of *sedation*. Commonly observed behavior changes include possible ventral tail curl, sleepiness, quietness, less responsive to human contact and purring.
2	*Euphoria*. Cats present specific behaviors such as rolling, playing with toys and invisible objects, purring, kneading with its forepaws and rubbing its head and body on the cage's door. The response to human contact is usually exaggerated. Some cats may meow. Friendly cat. Love-play bites.
3	Signs of *agitation* with increased locomotor activity. Behavior changes include staring, hyper-responsiveness, and vocalization but not accompanied by aggression or clear signs of fear and disorientation.
4	*Dysphoria*. A state of anxiety or restlessness usually accompanied by vocalization. Dysphoric behavior includes staring, agitation, and restless, pacing and sudden movements. Cats are hyper-responsive and become aggressive, fearful (“backing up into corners”) and/or disoriented.

Combined Discussion

Thermal antinociceptive investigation of a high-concentration buprenorphine and tapentadol yielded results of benefit to the acute management of pain in cats. Consistent thermal antinociception from high-concentration buprenorphine was demonstrated with all routes of administration. This is contrary to the previous conception that buprenorphine had a maximal or ceiling effect at lower doses. This assumption led to two decades of low-dose buprenorphine studies preventing advances and better understanding of this common drug. Thermal threshold testing also contradicted the paradigm that subcutaneous administration of buprenorphine is not a useful route of administration. This study showed that, at the doses used, the subcutaneous route of administration was superior to intravenous and buccal. Concurrent pharmacokinetics-pharmacodynamics provided a rationale behind this difference. Future investigation should include drug comparisons to quantify the magnitude of this effect to other opioids or formulations.

In both studies, dose was an important factor in the observed thermal antinociception. The increased buprenorphine dose yielded prolonged thermal antinociception compared to previous low-dose studies of the same routes of administration. The most striking of these differences is that of the subcutaneous route of administration. Compared to a previous paper of similar design using low-dose buprenorphine (65), the current study not only demonstrated prolonged antinociception but also increased magnitude of effect for the SC route. In the tapentadol paper, comparisons were made between the duration of thermal antinociception of tapentadol treated groups and the buprenorphine (positive control) group. This was an attempt to hypothesize clinical analgesic effect of tapentadol extrapolating from IM buprenorphine. Since the duration of clinical analgesia (if any) of tapentadol is unknown in cats, comparing duration of antinociception may be a crude appraisal. As mentioned in the literature review, with respect to superior analgesia between tramadol and pethidine in cats

undergoing ovariohysterectomy (103), a better analgesic is not one that simply lasts longer. While no magnitude of effect (inter-treatment) differences were detected between treated groups, the increase in mean thermal threshold of the buprenorphine group appears greater than that of either tapentadol group (Figure 1, Tapentadol paper). Perhaps then, as a preliminary dose-finding study, despite tapentadol producing thermal antinociception at both doses administered, the dose administered did not optimise the magnitude or duration of thermal antinociception. While the response observed in the study seemed appropriate, it would be a shame if future studies did not investigate alternative doses in an attempt to find this balance. One could look no further than the two studies presented in this thesis. The magnitude of thermal antinociception is similar between all buprenorphine groups (mean elevations in thermal threshold to between 50 and 51 °C) despite more than a 10-times higher dose being used in the SC group of the Simbadol study compared to the tapentadol study (Figure 1, Tapentadol paper and Figure 1, Simbadol paper). The difference in buprenorphine dose between these two studies seems to impact the duration of mean thermal antinociception more than it does magnitude. Fear (of "morphine-mania") or misconceptions about "ceiling" or maximal effects of buprenorphine likely delayed the investigation of increased doses and concentrations of this drug. A comparision which again nearly took nearly two decades since the first thermal antinociceptive evaluation of buprenorphine in cats to make (63).

The formulation of each drug facilitated administration of the treatments given in the current studies. The increased concentration of the formulation of Simbadol allowed for a smaller volume of injection for each treatment. At the licensed dose, 0.24 mg kg^{-1}, using low-concentrations of buprenorphine (0.3 mg ml^{-1}) would require a greater volume than the high concentration formulation (1.8 mg ml^{-1}) used in the current study (eg. for a 5 kg cat the volumes would be 4 ml vs. 0.67ml, respectively). While a complex two-phase absorption was observed when the drug was administered

subcutaneously in the current study, it is unclear the impact of the formulation on this mechanism. In Taylor et al.'s study of high-dose, a variety of buprenorphine concentrations were used (0.3 – 1.2 mg ml^{-1}) (66). The study found that regardless of the concentration used, when doses were 0.12 to 0.24 mg kg^{-1}, prolonged thermal antinociception (up to 30 h) was observed. It would be of interest to investigate whether differences exist with the higher formulation used in the present study compared to other commercially available concentrations. In the tapentadol study, the commercial form of the drug in an immediate-release format was used (50 mg Nucynta IR). As previously discussed, this was chosen as it is the only readily available format of the drug, making it presently available for use in veterinary practice. Unfortunately this formulation caused aversive behaviours in the cats, likely due to the drug's bitter taste. In future study, it would likely be of benefit to encapsulate the pill used. This would prevent direct contact between the drug and oral mucosa, hopefully preventing the stimulation of bitter taste receptors. In both studies, the formulation used are commercially available. This allows for a more direct dissemination of the results to clinical practice. The studies here support continued clinical use of Simbadol at its licensed dose and route of administration. Tapentadol in its current preparation needs further investigation and modification of formulation/drug delivery before it can be recommended for clinical use.

There are limitations to both studies which overlap. The presence of a cut-off thermal threshold (55 °C) produces artificially truncated data which potentially limits the ability to make inter-treatment comparisons. A method of dealing with right censored data (160) was attempted in the Simbadol study, however it did not produce a model of better fit. The mean thermal threshold in both studies was never equal to the cut-off value. However revision of the raw data, not presented or discussed in either study, showed 25 timepoints when cats reached cut-off thermal threshold values in the Simbadol study (SC n = 9, IV n = 7, and OTM n = 9) and 8 in the tapentadol study (BUP n = 7, LowTAP n = 1). It is unclear

whether the true thermal threshold (thermal threshold determined without safety cut-off) would be close to this threshold value or greatly increased from this value. In both studies no differences were detected between treated groups, but there remains a possibility a difference may exist. While this is a limitation of thermal threshold testing, it is essential to maintain a safety cut-off for both repeatability and for the welfare of the subjects tested. The inflammation associated with these burns would likely reduce thermal thresholds over time. Another limitation in both studies was the absence of pharmacodynamic modelling of norbuprenorphine and tapentadol. In developing the model in the Simbadol study, the absence of this information precluded its inclusion in the final model. When norbuprenorphine was included, models of better fit were not produced. Ultimately the final model produced may be incomplete, better understanding of the impact of norbuprenorphine on the thermal antinociception in cats is required to fill this gap of knowledge. In a similar manner, there is no available information on the pharmacodynamics of tapentadol in cats. In future study a combined pharmacokinetic-pharmacodynamic study should be performed to better elucidate the effects of tapentadol. Finally in each study only six cats were used in a cross-over manner. In both studies a pre-emptive power analysis was used to determine the appropriate number of cats used. In the Simbadol study, the results when comparing thermal thresholds to baseline and placebo were both similar and significant. This was despite the conservative statistical test using two-way ANOVA followed by Bonferroni's correction. The sample size in this study therefore seemed appropriate. In the tapentadol study, the same statistical tests were used. Large variation in the thermal threshold data was recognised as limiting comparisions in the placebo group to tapentadol-treated groups. Perhaps this is a result of insufficient sample size, or perhaps inappropriate statistical test. It has recently been suggested that linear mixed model analyses may be superior to ANOVA by reducing the frequency of type I errors (false positives) (169). In a thermal threshold crossover study of buprenorphine in American Kestrels (n = 12), a linear mixed model was able to detect that time-influenced thermal

antinoception over the study period, producing a type I error in the study (170). While this may simply be due to a flaw in thermal antinociceptive testing in the species, perhaps use of a larger population or a linear mixed model would have provided better insight to the results seen in the tapentadol study. Future studies would benefit from considering these limitations.

Conclusion

Antinociceptive studies allow for the better understanding of drugs already available, evaluating emerging therapeutics, and future discoveries. As was discussed in the attitudes and prevalence of analgesic administration, improvements have been made since 2000, but limitations now seem to be coming from an absence of dissemination of research findings to practitioners causing a deficiency in understanding the pharmacology of analgesics. While discussion and further refinement of instruments for the assessment of acute pain remain essential. The ultimate goal of these tools is developing methods of more efficiently detecting painful animals so as to be able to better treat and prevent pain. Detection, while important, is futile in the absence of the ability to treat pain. A specific shortfall discussed was the absence of post-operative drug administration and at home analgesia being provided to cats. Both treatments investigated in this thesis allow for either prolonged analgesia (Simbadol) or continued oral analgesics (tapentadol). Both of which would allow cats to return home faster and more comfortably. While treatments in hospital remain limited for cats (compared to dogs), continued pain management at home remains problematic. Simbadol is already licensed in the United States, and its continued use perioperatively is supported by the prolonged pharmacokinetic-pharmacodynamic modelling provided in this thesis. Tapentadol needs further refinement and study before it can be used as a clinical analgesic in feline medicine, but as a first evaluation to its potential analgesic effects in cats, the drug shows some promise. Developing this knowledge base is essential to the understanding of feline clinical pain.

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