Evaluation of analgesic effect and absorption of buprenorphine after buccal administration in cats with oral disease

Thaleia- Rengina Stathopoulou,[1] Maria Kouki,[2]Bruno H. Pypendop,[3] Atholl Johnston,[4] Serafeim Papadimitriou,[2] Ludovic Pelligand[1]

[1] Clinical Services and Sciences, Royal Veterinary College, , Hawkshead Lane, Hatfield, UK AL97TA

[2] Aristotle University of Thessaloniki School of Veterinary Medicine, Thessaloniki GR

[3] Surgical and Radiological Science,School of Veterinary Medicine, University of California, One Shields Avenue, Davis,CA, USA 95616

[4] Queen Mary University of London, and Analytical Services International, St George's, University of London, Cranmer Terrace, London, SW17 0RE, UK

Keywords: feline, buprenorphine, gingivostomatitis, buccal administration, analgesia

Corresponding author: Thaleia- Rengina Stathopoulou DVM, MRCVS, MVetMed, Royal Veterinary College, Clinical Services and Sciences, Hawkshead Lane, Hatfield, UK AL97TA

Email: tstathopoulou@rvc.ac.uk

Abstract

Objectives: To evaluate the analgesic effect and the absorption of buprenorphine after buccal administration in cats with oral disease.

Methods: Six adult client-owned cats with chronic gingivostomatitis (weighing 5.1kg +/- 1.1kg) were recruited for a randomised, prospective, blinded, saline controlled crossover study. Pain scores, dental examination, stomatitis score and buccal pH measurement were conducted on day 1 under sedation in all cats. On day 2, animals were randomized in two groups and administered one of the two treatments buccally (group A received buprenorphine 0.02mg/ kg and group B received 0.9% saline) and vice versa on day 3. Pain scores and food consumption were measured at 30, 90 and 360 mins after the administration of buprenorphine. Blood samples were taken at the same time and plasma buprenorphine concentration was measured by liquid chromatography- mass spectrometry. Data were statistically analysed as non-parametric and level of significance was set as $P<0.05$.

Results: There were no major side effects after buprenorphine administration. Buccal pH values ranged between 8.5-9.1 and stomatitis disease activity index 10-22 (17.8+/- 4.5) with the scale ranging from 0-30. The maximum buprenorphine plasma concentration (14.8 ng/ ml) was observed 30 minutes after administration and there was low interindividual variability. There was a significant difference between baseline pain scores compared to pain scores after buprenorphine (*$P<0.05$)* and between the saline and buprenorphine group at 30 mins ($p=0.04$) and 90 mins ($P=0.04$). There was also a significant effect of stomatitis index on pain score. Regarding the pharmacokinetic parameters, cats with stomatitis showed lower bioavailability and shorter absorption half-life after buccal administration of buprenorphine compared to normal cats in previous studies.

Conclusion and clinical relevance: Buccal administration of buprenorphine in cats with gingivostomatitis produces an analgesic effect and low interindividual variability in plasma

concentration and it can be incorporated in the multimodal analgesia plan of cats with gingivostomatitis.

Introduction

Pain management is the cornerstone of veterinary practice and constitutes not only a professional obligation but also a way to enhance animals' quality of life. In the recent years, there has been increased interest into pain assessment and management in cats that have been historically undertreated for pain compared to other species.[1-3]

Opioids play an important role in the multimodal approach to pain management in cats with buprenorphine being one of the drugs most widely used.[4] Buprenorphine, a highly lipophilic semi synthetic partial agonist at μ (mu) opioid receptors, is considered a unique drug with complex pharmacology.[5] It is the most commonly used opioid in small animal practice in the UK,[1] being also widely used in the vast majority of continental Europe, Australia and South Africa.[2, 6] Common morphine and hydromorphone side effects such as nausea, vomiting and salivation are rarely seen after buprenorphine[7]. This advantage, alongside with its efficacy and long duration of action[8, 9] justifying its popularity.

In feline patients, studies have proven that the buccal route of administration (OTM) of buprenorphine shows a bioavailability similar to the intravenous (IV) and intramuscular (IM) routes.[10-12] According to Robertson et al (2005),[10] the analgesia provided by the buccal administration is comparable to the one of alternative routes. However, among others the study from Giordano et al. (2010)[13] demonstrated inferior analgesic effect of the buccal route compared to IV and IM after ovariectomy and Santos et al[14] found less sedative effect after buccal administration of dexmedetomidine and buprenorphine compared to IM route.

The systemic absorption of buprenorphine after buccal administration depends on the mucosal pH. Buprenorphine is a weak base pKa (8.24) and therefore an alkaline environment, such as the cat's oral

72 cavity with pH between 8 and 9, favours its unionised form and enhances its bioavailability by avoiding
73 the first pass elimination.[10, 15]

74 The blood-sampling site has also an impact on buprenorphine concentration–time profile. Following
75 buccal administration in cats, venous blood sampling from a jugular site is not an acceptable substitute
76 for arterial blood sampling,[16] as the perfusion of the oral mucosa drains from the same vein resulting
77 in overestimation of drug's systemic availability. The above can explain the high bioavailability of
78 buprenorphine (116%) found in previous studies[10] following buccal administration as external jugular
79 was used for sampling.

80 Severe inflammation of the oral cavity, described with the term gingivostomatitis,[17] is a multifactorial
81 disease often seen in feline patients and it can be a chronic, devastating and painful condition. The exact
82 aetiology of the condition is unknown, with environmental factors, bacterial and viral infection being
83 most often implicated,[18] though neoplastic, autoimmune, developmental and congenital conditions can
84 be recognised as co-factors as well. Clinical signs include oral pain, halitosis, dysphagia, anorexia and
85 weight loss, while some cats are euthanized because of poor quality of life.[19] Treatment of
86 gingivostomatitis is mainly symptomatic and involves antibiotics, corticosteroids, opioids, non-
87 steroidal anti- inflammatory agents (NSAIDs), laser thermoablation, cyclosporine, oral surgery and
88 tonsillectomy. Plasmapheresis, human immunoglobin and feline interferon omega have also been
89 used.[20] It is not known whether the presence of gingivostomatitis affects the saliva pH and thereby the
90 absorption and the bioavailability of buprenorphine after buccal administration.

91 We designed a saline-controlled crossover efficacy and pharmacokinetic study in cats with
92 gingivostomatitis to assess whether the presence of oral inflammation in the oral cavity affected the
93 rate of oral transmucosal absorption, the overall systemic uptake and the analgesic efficacy of
94 buprenorphine. Our alternative hypothesis was that there would be a difference in analgesia between
95 the buprenorphine and saline groups after buccal administration, with buprenorphine providing
96 superior analgesia. The prevalence of feline gingivostomatitis in the UK is 0.7%, but appears to be much

97 higher (13.1%) in studies in United States and Southern Europe.[18] Due to the higher prevalence of oral
98 diseases in Southern Europe we recruited patients at the Aristotle University (Greece). [21]

99

100 **Materials and methods**

101 The study was designed as a randomised, prospective, saline-controlled, blinded crossover study. The
102 design is summarised in Figure 1. Ethics approval was granted by the Aristotle University of
103 Thessaloniki, Greece and written owner consent was obtained for this clinical trial.

104 Six client owned adult cats, ASA (American Society of Anaesthesiologists) physical status I or II, with
105 evidence of oral inflammation were included in the study. No abnormal finding other than signs of
106 gingivostomatitis was detected during physical examination. The cats had not received any opioids
107 five days prior their arrival. Concurrent NSAIDs and/or antibiotics course were not exclusion criteria.

108 Allocation of the first treatment was randomised by the means of sealed envelopes containing the
109 number of each cat. The first three chosen by a blinded investigator were assigned to group A and the
110 rest to group B.

111 On day 1, physical examination was performed and baseline pain scores were recorded, according to a
112 modified BOTUCATU pain scale[22] (range from 0 to 27, Appendix 1). All cats were, subsequently,
113 sedated with 0.02mg/kg medetomidine intramuscularly (IM) (Sedastart, Animalcare). During sedation,
114 oral pH was measured with pH stripes (Simplex Health), oral lesions were staged and mapped using a
115 dental examination form and stomatitis disease activity index[23] (Appendix 2, 3). An intravenous
116 peripheral catheter (22G, 25mm. Jelco, Smiths Medical) was placed in a cephalic vein to facilitate blood
117 sampling and to decrease any additional discomfort for the patients. Sedation was reversed with 0.05
118 mg/kg of atipamesole (Sedastop, Animalcare) IM. The catheters were flushed every 4 hours with 2ml
119 of heparinised saline to secure their patency and a light bandage was placed for protection.

On day 2, the cats from group A received 0.02 mg/kg of buprenorphine (group BUP, Buprecare, Animalcare) by buccal route and group B received equal volume of 0.9% saline (group SAL, Vetivex1, Dechra Animal Products) by the same route. Both treatments were administered with a 1 ml syringe (B. Braun medical) in the right cheek pouch by the principal investigator (TS) that was blinded to treatment allocation. Cats were assessed for the presence of hypersalivation, mydriasis, grooming activity and food consumption (yes/no) 30, 90 and 360 minutes following the treatment administration. Pain assessments were performed by the same investigator at the same times using the same scale (Modified BOTUCATU pain scale) as for baseline and for day 1.

Blood samples were collected by the assessor (MK), who was aware of treatment allocation, 30, 90 and 360 mins after buprenorphine buccal administration, but not after saline administration. Following pain scoring, samples were taken from the cephalic catheter after 2 ml of blood were aspirated to ensure a non-diluted blood sample. One ml of blood was collected in potassium EDTA blood tubes (Vetlab). The samples were centrifuged (Centrifuge Heraeus -Christ GmbH Osterode, Harz Simplex, GE) for eight minutes at 4039g within 30minutes after collection. The plasma (0.5 to 0.7 mL) was separated and stored in -80 ^{0}C (Model 725, Thermo-Forma) in labelled Eppendorf tubes.

On day 3, the alternative treatment was administered, with group A receiving the 0.9% saline treatment and group B receiving 0.02 mg /kg buprenorphine buccally, and the same procedure as on day 2 was followed.

Plasma samples were shipped to the UK on dry ice and analysed by St Georges University in London. Plasma buprenorphine was measured using a validated liquid chromatography – tandem mass spectrometry method (LC/MS/MS),[24] initially validated in man. The method was revalidated for feline plasma and met standards for sensitivity, linearity, precision, accuracy and stability generally accepted in bioanalytical chemistry.[25] The lower limit of quantification of the assay was 0.025 ng/mL.

Population pharmacokinetic modelling was performed with Phoenix NMLE®, version 1.3, Certara (Princeton, NJ, USA). Briefly, a two-compartmental model was built to be simultaneously fitted to the

plasma buprenorphine concentration-time data from the present study (sparse sampling) and those from a previously published study performed in healthy cats administered the same dose of buprenorphine intravenously and by the buccal route (rich sampling).[26]Full description of the joint population PK model is provided in Appendix 4. The goal of including external IV and buccal route data in the PK model was to leverage information (clearances and volumes of distribution assumed to be distributed similarly in stomatitis and healthy cats) and increase the number of degree of freedom, as done in Pelligand et al.[27]This allowed the fitting of the most likely plasma concentration time-curve in sparsely sampled cats and the estimation of bioavailability and absorption rate constant in the study with stomatitis cats.

Statistical analysis

A commercially available programme was used for the statistical analysis (IBM SPSS Statistics 22). Data distribution was assessed for normality graphically and by the results of Kolmogorov -Smirnov statistic. Due to violation of the assumption of normality, the Wilcoxon matched -pairs signed rank test was used was used to compare pain scores obtained as baseline, after saline and after buprenorphine administration and at 30, 90 and 360 mins. The level of significance was set as $P < 0.05$. Pharmacokinetic parameters distributions were compared between cats with gingivostomatitis and normal cats from a previous study[26] using the Mann-Whitney U-test.

Correlation analysis was used to describe the strength and the direction of the linear relationship between variables. Spearman Rank Order Correlation was used for non-parametric data testing of correlation between stomatitis activity index score and both pH and pain scores. Food consumption (yes/no) was tested at each time point with a Fisher's exact test.

Results

Six, client owned, adult cats were included in this clinical study, four male neutered and two female neutered. Their age ranged from 7 to 10 years (mean 9.1years) and their body weight ranged from 4 to 7 kg (mean 5.1kg). Two of the cats were receiving antibiotics, one of them was also receiving meloxicam for their stomatitis, and the last dose was given 48 h before presentation.

No adverse effects were noted in this study except hypersalivation in two of the cats after the administration of buprenorphine that resolved within minutes. All cats developed mydriasis within 5 minutes after the administration of buprenorphine, except in one cat in which this could not be evaluated due to bilateral enucleation. Mydriasis persisted for several hours after buprenorphine administration. Mydriasis does not correlate with analgesia or antinociception.[9]

The oral pH values ranged from 8.5 to 9 and the stomatitis disease activity index ranged from 10 to 22 (mean 17.8+/- 4.5). Three of the cats had partial mouth extractions of the premolar and molar teeth and three had previously full mouth extractions. However, that was completed at least a year before presentation. The positive correlation between the variables of pH and stomatitis disease index and pH was not significant (P= 0.152).

Food consumption evaluation was part of the total pain scores. Small amount of wet and dry food was offered repeatedly at these timepoints Overall at 30 mins, all cats in the buprenorphine groups ate some wet food compared to 2 in the saline groups (P= 0.061). At 90 minutes, cats treated with buprenorphine had a significantly higher chance to eat than the ones with saline (6 cats for buprenorphine vs 1 saline, P = 0.0152). There was no difference at 360 minutes (2 cats for buprenorphine vs 3 cats for saline, P = 0.54). None of the cats started eating dry food at any time point.

Pain scores (figure 2) decreased significantly with buprenorphine (BUP) and saline (SAL) administration compared to baseline (BSL, P = 0<.001). When testing each time point, the pain scores for the BUP group were significantly lower than BSL at 30 mins (P = 0.0007) and 90 mins (P=0.011) and

were significantly lower than SAL at 30 mins (P=0.04) and at 90mins (P=0.04), but not at 360 mins (P=0.09). Linear mixed model also revealed a significant effect of stomatitis index score on pain score (P=0.001).

The time of maximum buprenorphine plasma concentrations in cats with gingivostomatitis was at the 30-mins blood sample when concentrations ranged from 274 to 1 621 ng/ mL. One cat (10-year female neutered 4.2kg cat treated with clindamycin, meloxicam, dental score 18) had a very high plasma concertation (84 979 ng/mL). This data point was excluded from the analysis on the basis that such high plasma concentrations were not reached even in early 1 and 3-minute samples after IV administration[22] and is likely to result from contamination of the sample. The most likely buprenorphine plasma concentration-time plot for the cats with gingivostomatitis is shown in figure 3. For all parameters listed below, the inter-individual variability (IIV %) is reported immediately following each estimate where appropriate. Pharmacokinetic parameter (Table 1) estimates for clearance, intercompartmental clearance, volume of distribution of the central and peripheral compartment displayed low inter-individual variability even in a mixed group and were close to values previously reported.[26]

The pharmacokinetic parameters are presented in Table 1 and described in Appendix 4 (figure 4).

Discussion

During this study, no side effects were identified, except hypersalivation in two cats. All cats, except the one that had bilateral enucleation, developed mydriasis.

There is a lack of evidence in veterinary literature on whether oral inflammation affects buccal pH values. The values of buccal pH in our study ranged between 8.5 and 9.1 and are relatively lower compared to Robertson's study[10] (p H =9.0) but higher compared to Hedges's[26] (pH =8.0). A correlation between the buccal pH and the stomatitis disease activity index was not identified. An increase in pH

is associated with increased salivation in humans[28] due to an increase of sodium and bicarbonate.[29] In cats, stomatitis is often related with sings of hypersalivation.[17]

Cats showed increased appetite at 30 and 90 mins after buprenorphine administration, which could be due to additional analgesia or euphoria. An increase in food consumption is a rare manifestation of pain in cats.[30] None of the cats ate dry food which could be due to insufficient pain relief or to preference as cats were offered simultaneously wet and dry food. The influence of a hospital environment should also be considered. Some cats remain unresponsive and passive in new environments or can be hyperactive.[31, 32] Increased food intake would be an important benefit, considering that compromised nutrition is one of the most important problems encountered with gingivostomatitis.[33]

Pain scores following buprenorphine administration were lower than at baseline and following saline administration. This can be attributed to pain relief as well as the euphoria produced by opioids. In addition, local effect of buprenorphine needs to be considered since a study in humans found that buprenorphine decreased the postoperative pain and increased the duration of analgesia when added to the inferior alveolar nerve block for dental surgery, compared to intramuscular administration.[34] The fact that the pain scores were lower after saline administration compared to baseline, could be attributed to acclimatisation in the new environment, as well familiarisation with the pain scoring process and the evaluator. The effect of stomatitis index on pain score was expected, as cats with more severe stomatitis are expected to be more painful. Our alternative hypothesis that pain scores would be lower following buprenorphine than following saline was confirmed, as there was a significant difference at 30 and 90 mins. The plasma buprenorphine concentration at 360 mins may have been inadequate to provide analgesia. In any case, the results may suggest that the duration of effect of buprenorphine at the dose used may be shorter than previously reported.

The time of maximum plasma buprenorphine concentration was 30 minutes following administration and pharmacokinetic analysis showed low interindividual variability with values close to those obtained by Hedges et al[26] in cats with normal oral mucosa. Transmucosal drug absorption, though,

241 depends on many different factors like its concentration and the mucosal contact time.[35] Buprenorphine
242 was administered in the cheek pouch but the degree of inflammation on the specific area could not be
243 determined. Inflammation-induced vasodilation could have led to an earlier maximum concentration
244 that we were unable to detect as our first blood sample was at 30 min. In addition, cats might have
245 swallowed or spitted a portion of the drug, as they were sensitive in handling of the head and did not
246 tolerate their mouth to be held closed after treatment. The formulation used in this study was a multi-
247 dose vial (Buprecare, Animalcare,) containing 0.135% chlorocresol as a preservative and it is possible
248 that the preservative free buprenorphine could be better tolerated, while there is no difference
249 regarding their pH among the formulations.[36] The multi-dose vials are commonly used in practice due
250 to cost effectiveness and easy usage and storage.

251 In our study, the mean absorption half-life of buprenorphine was longer compared to Hedges et al. ,[26]
252 which included normal cats. However, there was no significant difference in bioavailability, although
253 the present study may have been underpowered to detect a difference. The difference in absorption
254 rate could be due either to the different formulations of buprenorphine that were used in the two
255 studies, to the actual modalities of administration or an effect of the higher pH and the presence of
256 gingivostomatitis.

257 The study had several limitations. The lack of a sensitive and validated pain scale for oral pain is a
258 major limitation. UNESP-Botucatu scale is the only pain scoring system for cats with published data on
259 reliability, validity and sensitivity[30] and we modified it for oral pain using the oral cavity as the painful
260 reference point and the head and neck area as the surrounding tissues. We omitted the blood pressure
261 measurement because it could be stressful and unreliable when repeated in frequent intervals. The
262 maximum point of our pain scale was 27 instead of 30 in the original scale. The small sample size is
263 another limitation that could have affected our statistical analysis. Furthermore, the use of historical
264 data for modelling in lieu constitutes one more limitation, as is the use of data from another study that
265 were obtained under different conditions and analysed using a different assay, despite that they were

remodelled using the study population model. The fact that one of the cats was receiving meloxicam constitutes another limitation. However, the last dose was given 48h before presentation and the baseline pain score of this cat that could have been potentially affected was similar to the rest of the cats. In addition, there is no possibility that co-administration of NSAIDs interferes with the quantitative analysis of buprenorphine by liquid chromatography mass spectrometry because of the high specificity of the method. Finally, the values of buccal pH were also obtained on day 1 after the administration of medetomidine that could have also affected the value, so we are not aware of the actual pH value on the time of buprenorphine administration.

Conclusion

Buccal administration of buprenorphine in cats with gingivostomatitis produces an analgesic effect and has low interindividual variability regarding plasma concentration. Further studies are needed to elucidate the role of oral inflammation on buccal drug absorption in cats as well as the potential benefit and appropriateness of opioids compared to the current analgesia alternatives such as NSAIDs. Furthermore, considering that sublingual buprenorphine constitutes an effective treatment of chronic pain in humans [37] and that subcutaneous buprenorphine prevented hyperalgesia in cats,[38] studies on the long-term use of buprenorphine by the buccal route in cats with chronic gingivostomatitis and the evaluation of the potential benefits and side effects would be of clinical interest.

Supplementary material

Appendix 1: UNESP-Botucatu Multidimensional Composite Pain Scale for assessing postoperative pain in cats, modified to assess oral pain.

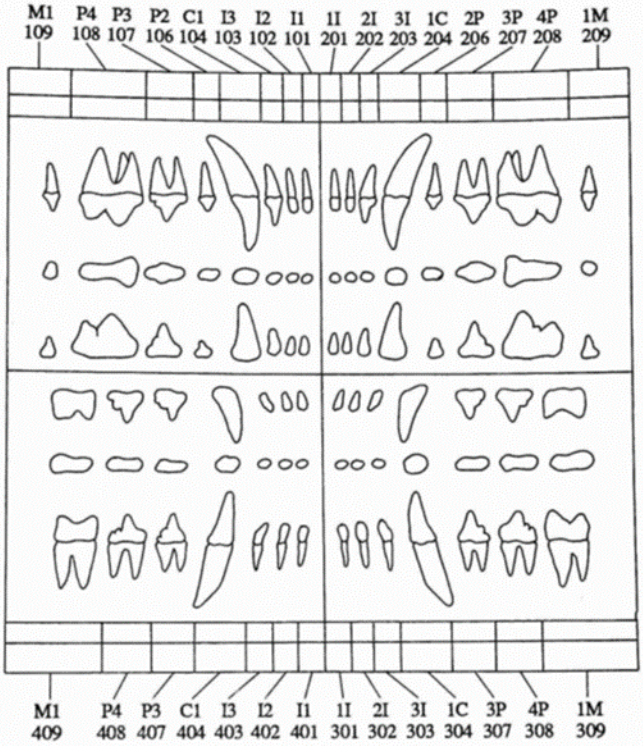
	Subscale 1: PAIN EXPRESSION (0 – 12)	
Miscellaneous behaviour	Observe and mark the presence of the behaviours listed below **A** - The cat is laying down and quiet, but moving its tail **B** - The cat contracts and extends its thoracic limbs and/or contracts its neck muscles **C** - The cat's eyes are partially closed (eyes half closed) **D** - The cat licks and/or bites the surgical wound • All above behaviours are absent • Presence of one of the above behaviours Presence of two of the above behaviours Presence of three or all of the above behaviours	A B C D 0 1 2 3
Reaction to palpation of the area around the mouth cavity	• The cat does not react when the mouth is touched or pressed; • The cat does not react when the area around the mouth is touched, but does react when it is pressed. It may vocalize and/or try to bite • The cat reacts when the mouth is touched and when pressed. It may vocalize and/or try to bite • The cat reacts when the observer approaches the mouth. It may vocalize and/or try to bite The cat does not allow palpation around mouth cavity	0 1 2 3
Reaction to palpation of the head	• The cat does not react when the head is touched • The cat does not react when the head and neck are touched, but does react when it is pressed. The neck is tense • The cat reacts when the head and neck are touched and when pressed. The neck is tense • The cat reacts when the observer approaches the head It may vocalize and/or try to bite The cat does not allow palpation of the head and neck	0 1 2 3
vocalisation	• The cat is quiet, purring when stimulated, or miaows interacting with the observer, but does not growl, groan, or hiss • The cat purrs spontaneously (without being stimulated or handled by the observer) • The cat growls, howls, or hisses when handled by the observer (when its body position is changed by the observer) • The cat growls, howls, hisses spontaneously (without being stimulated or handled by the observer)	0 1 2 3

	Subscale 2: PSYCHOMOTOR CHANGE (0 – 12)	
posture	• The cat is in a natural posture with relaxed muscles (it moves normally)	0
	• The cat is in a natural posture but is tense (it moves little or is reluctant to move)	1
	• The cat is sitting or in sternal recumbency with its back arched and head down; or The cat is in dorso-lateral recumbency with its pelvic limbs extended or contracted	2
	The cat frequently alters its body position in an attempt to find a comfortable posture	3
comfort	• The cat is comfortable, awake or asleep, and interacts when stimulated (it interacts with the observer and/or is interested in its surroundings)	0
	• The cat is quiet and slightly receptive when stimulated (it interacts little with the observer and/or is not very interested in its surroundings)	1
	• The cat is quiet and "dissociated from the environment" (even when stimulated it does not interact with the observer and/or has no interest in its surroundings) The cat may be facing the back of the cage	2
	The cat is uncomfortable, restless (frequently changes its body position), and slightly receptive when stimulated or "dissociated from the environment" The cat may be facing the back of the cage	3
activity	• The cat moves normally (it immediately moves when the cage is opened; outside the cage it moves spontaneously when stimulated or handled)	0
	• The cat moves more than normal (inside the cage it moves continuously from side to side)	1
	• The cat is quieter than normal (it may hesitate to leave the cage and if removed from the cage tends to return, outside the cage it moves a little after stimulation or handling)	2
	The cat is reluctant to move (it may hesitate to leave the cage and if removed from the cage tends to return, outside the cage it does not move even when stimulated or handled)	3
attitude	Observe and mark the presence of the mental states listed below **A - Satisfied:** The cat is alert and interested in its surroundings (explores its surroundings), friendly and interactive with the observer (plays and/or responds to stimuli) *The cat may initially interact with the observer through games to distract it from the pain. Carefully observe to distinguish between distraction and satisfaction games	A
	B - Uninterested: The cat does not interact with the observer (not interested by toys or plays a little; does not respond to calls or strokes from the observer) * In cats, which don't like to play, evaluate interaction with the observer by its response to calls and strokes	B
	C - Indifferent: The cat is not interested in its surroundings (it is not curious; it does not explore its surroundings) * The cat can initially be afraid to explore its surroundings. The observer needs to handle the cat and encourage it to move itself (take it out of the cage and/or change its body position)	C
	D - Anxious: The cat is frightened (it tries to hide or escape) or nervous (demonstrating impatience and growling, howling, or hissing when stroked and/or handled)	D
	E - Aggressive: The cat is aggressive (tries to bite or scratch when stroked or handled)	E
	Presence of the mental state A	0
	Presence of one of the mental states B, C, D, or E	1
	Presence of two of the mental states B, C, D, or E	2
	Presence of three or all of the mental states B, C, D, or E	3

Subscale 3: PHYSIOLOGICAL VARIABLES (0 – 3)		
Appetite	• The cat is eating normally • The cat is eating more than normal • The cat is eating less than normal • The cat is not interested in food	0 1 2 3
TOTAL SCORE (0 – 27)		

Appendix 2: feline dental chart (Holmstrom S, Frost P and Eisner E. *Veterinary dental techniques: for the small animal practitioner.* 2nd ed. W. B. Saunders Company, 1998, pp17-18)

Pet Clinic
Feline Dental Treatment Chart



Remarks and Diagnosis: _____

_____

_____

Radiographic Evaluation and Assessment: _____

_____

_____

Treatment Summary and Plan: _____

_____

_____

Client Instructions: _____

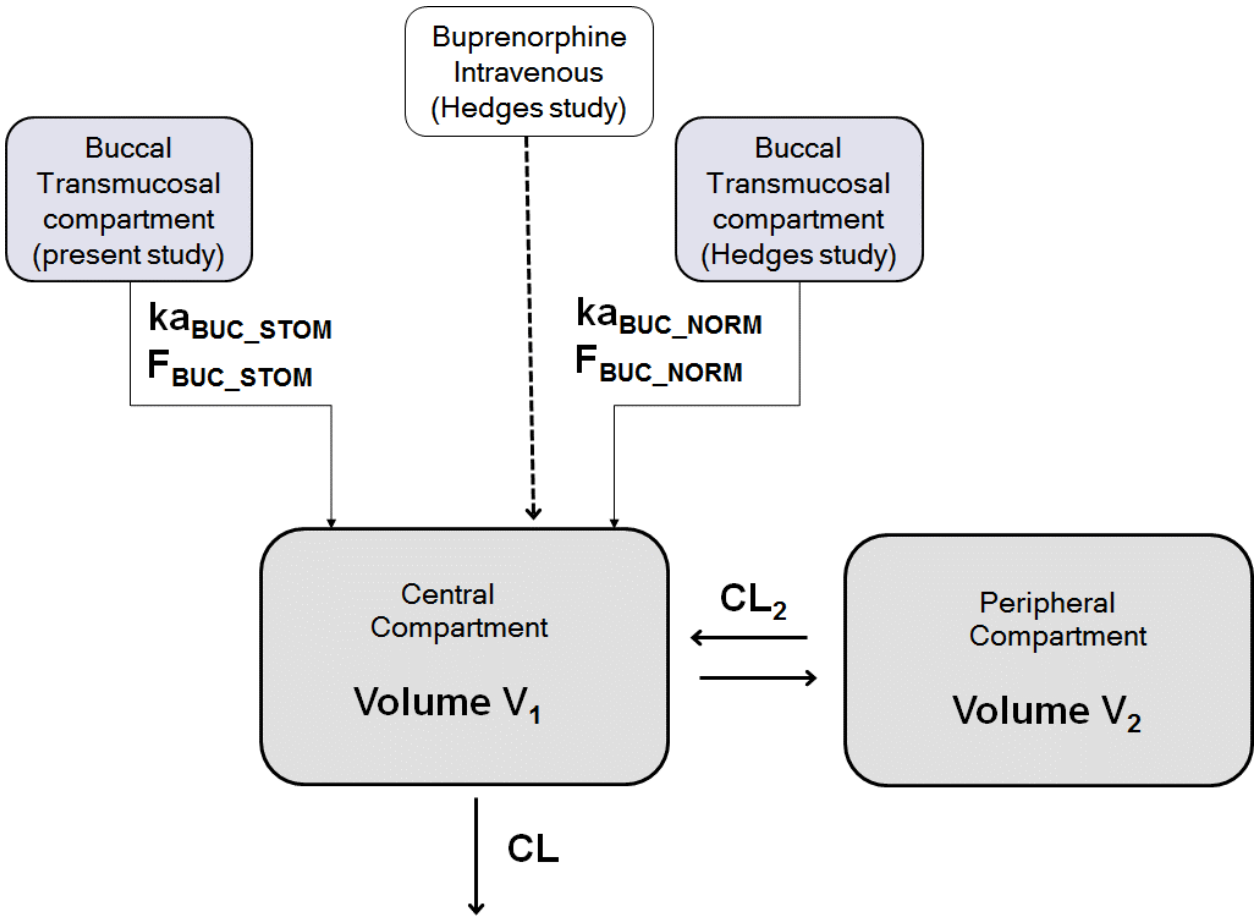
_____

Appendix 3: Stomatitis disease activity index score. [23]

STOMATITIS DISEASE ACTIVITY INDEX	0	1	2	3
Owner's evaluation(average appetite/activity/grooming)				
Owner's evaluation perceived comfort				
Maxillary buccal mucosal inflammation				
Mandibular buccal mucosal inflammation				
Maxillary attached gingival inflammation				
Mandibular attached gingival inflammation				
Inflammation lateral to palatoglossal folds				
Molar salivary gland inflammation				
Oropharyngeal inflammation				
Lingual and/or sublingual inflammatio				
Total score(maximum 30)				

Appendix 4: Population pharmacokinetic-pharmacodynamic modelling

A classic two-compartment model with first order absorption was the starting point for compartmental modelling of the buccal route. We used the raw data from a previous publication (Hedges et al. 2013 with 6 healthy cats receiving buprenorphine IV and buccally) to support the PK modelling in clinical cats from which only 3 blood samples were taken.



Parameters: CL: body clearance, CL_2: inter-compartmental clearance, V_1: central volume of distribution, V_2: peripheral volume of distribution, ka_{BUC_STOM}: absorption rate constant in cats with stomatitis, ka_{BUC_NORM}: absorption rate constant in normal cats, F_{BUC_STOM}: bioavailability in cats with stomatitis, F_{BUC_NORM}: bioavailability in normal cats.

Goodness of fit:

For each Phoenix NMLE run, plots of goodness of fit were prepared [39]. The nested candidate models were compared on the basis of their biological plausibility, prediction based diagnostics (PRED, IPRED,), residual-type diagnostics (RES and IRES) and numerical diagnostics (minimisation of the Objective Function Value (OVF) statistically tested with the Likelihood Test Ratio (was LRT performed, deltaOVF >6.64; P<0.01, df = 1, or alternatively use the Akaike Information Criterion, AIC)

as well as measures of model stability and adequacy (convergence, precision of the parameters estimates).

Statistical description of the model:

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

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

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 bounded between 0 and 1 (typically bioavailabilities, noted F) were expressed and estimated in the model after a logit transform and the typical value of F (θ_F) was back-converted as in equation 2 to yield 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 parameter was 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 the simulated (n=5000) plasma concentrations with the observed data (+/- 5^{th} and 95^{th} percentiles).

PK modelling

Base model development for the buccal administration

First, a 2 compartment model was written to fit simultaneously the IV and the buccal route to allow estimation of the physiological PK parameters common to the three routes of administration (namely CL, the total body clearance; V, the volume of the central compartment; CL_2, the intercompartmental clearance and V_2, the volume of the peripheral compartment), as well as the buccal absorption rate constants (ka_{BUC}) and the absolute buccal bioavailabilities (F_{BUC}). The typical value θ_j and individual η_{ij}

were fixed to reduce the number of parameters to estimate in the modelling of the complex SC absorption.

Table1: Comparison of rival models for joint IV and buccal buprenorphine model and selection of best model

Joint model	**OFV (-2LL)**	**AIC**	**Comment**
Combined IV and buccal, proportional error	221	255	Best model
Combined IV and buccal, additional error	443	477	

PK parameters estimates (see also Table 1 in manuscript):

The two routes of administration shared four central PK parameters; clearance (CL = 1.26 L/ kg / hour, 1.1%), volume of distribution of the central compartment (V_1 = 0.65 L/kg, 0.9%), intercompartmental clearance (CL_2 = 1.19 L /kg/hour, 2.3%) and peripheral volume of distribution (V_2 = 6.96 L/ kg, 7.8%) with a common proportional residual error term.

For PK parameters specific to the buccal treatment, the mean bioavailability in the cats with gingivostomatitis with the current formulation (Buprecare®, animalcare) was 19.5% (IIV 65.7%) compared to 28.8% (IIV 19.6%) in the normal cats in the study by Hedges et al[26], in which another formulation was used (Buprenex® Injectable; Reckitt Beckiser Pharmaceuticals). This difference was not significant (P = 0.31). The absorption rate constant in cats with gingivostomatitis was 0.57/hour, yielding an absorption half-life of 1.2 hours. For the normal cats in the study by Hedges et al. [26], the absorption rate constant was 1.39/hour, yielding a significantly shorter absorption half-life of 0.49 hours.

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: individual observed concentrations and model predictions vs time,

Fig suppl. 1 (observed values vs population predictions PRED)

Legends: CObs_A_IV: buprenorphine after IV administration (Hedges et al, 2013), CObs_B_OTM: buprenorphine after buccal administration (Hedges et al, 2013), CObs_C_OTM: buprenorphine after buccal administration (present study), DV = dependent variable (observed value), PRED = population predictions, IPRED = individual predictions

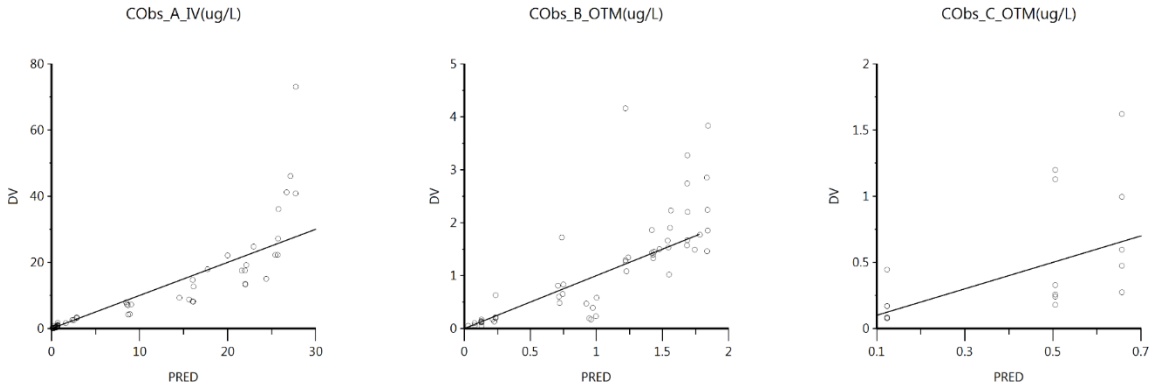


Fig suppl. 2 (observed values vs individual predictions IPRED)

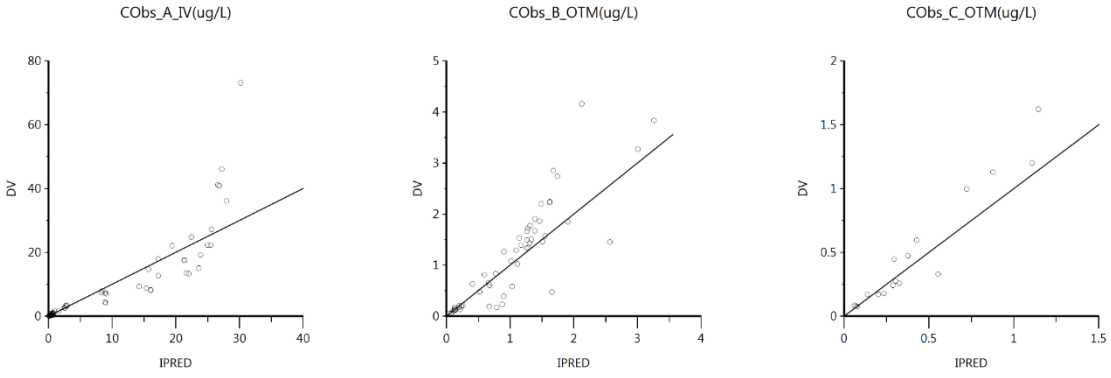


Fig suppl. 3 (conditional weighted residuals vs time after dose)

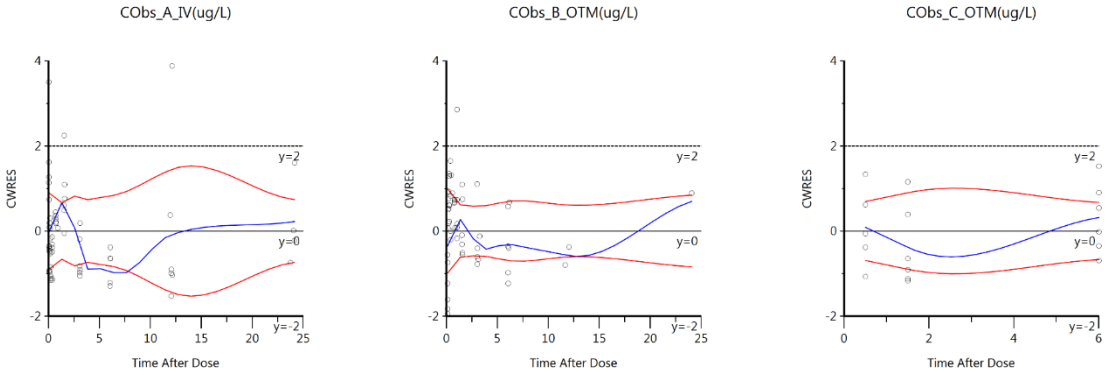


Fig suppl. 4 (conditional weighted residuals vs population prediction)

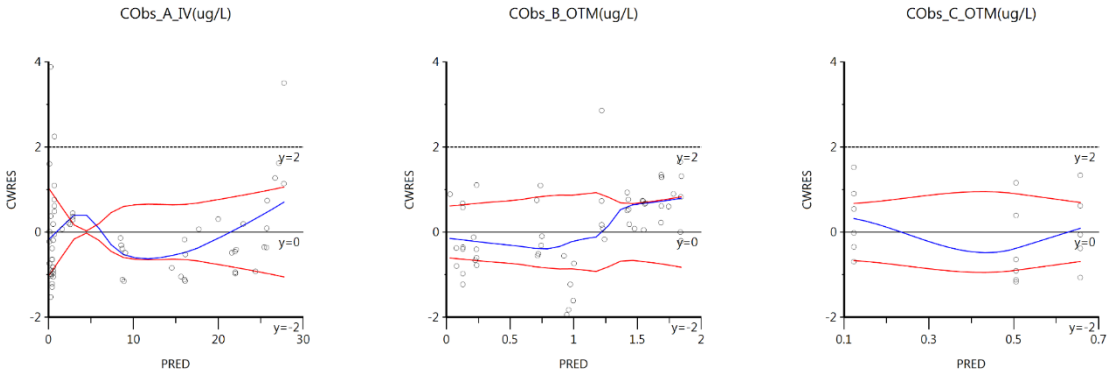
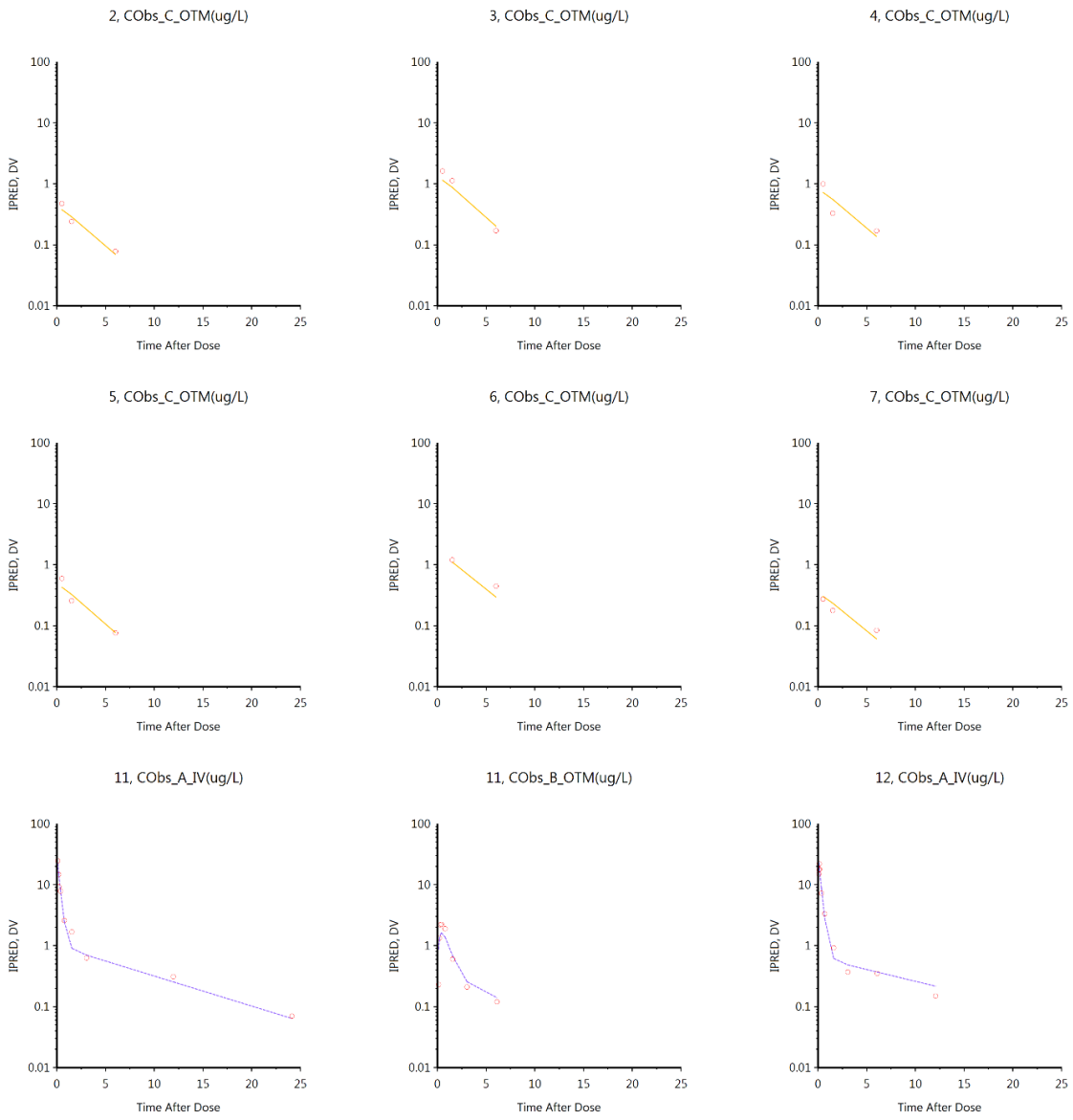
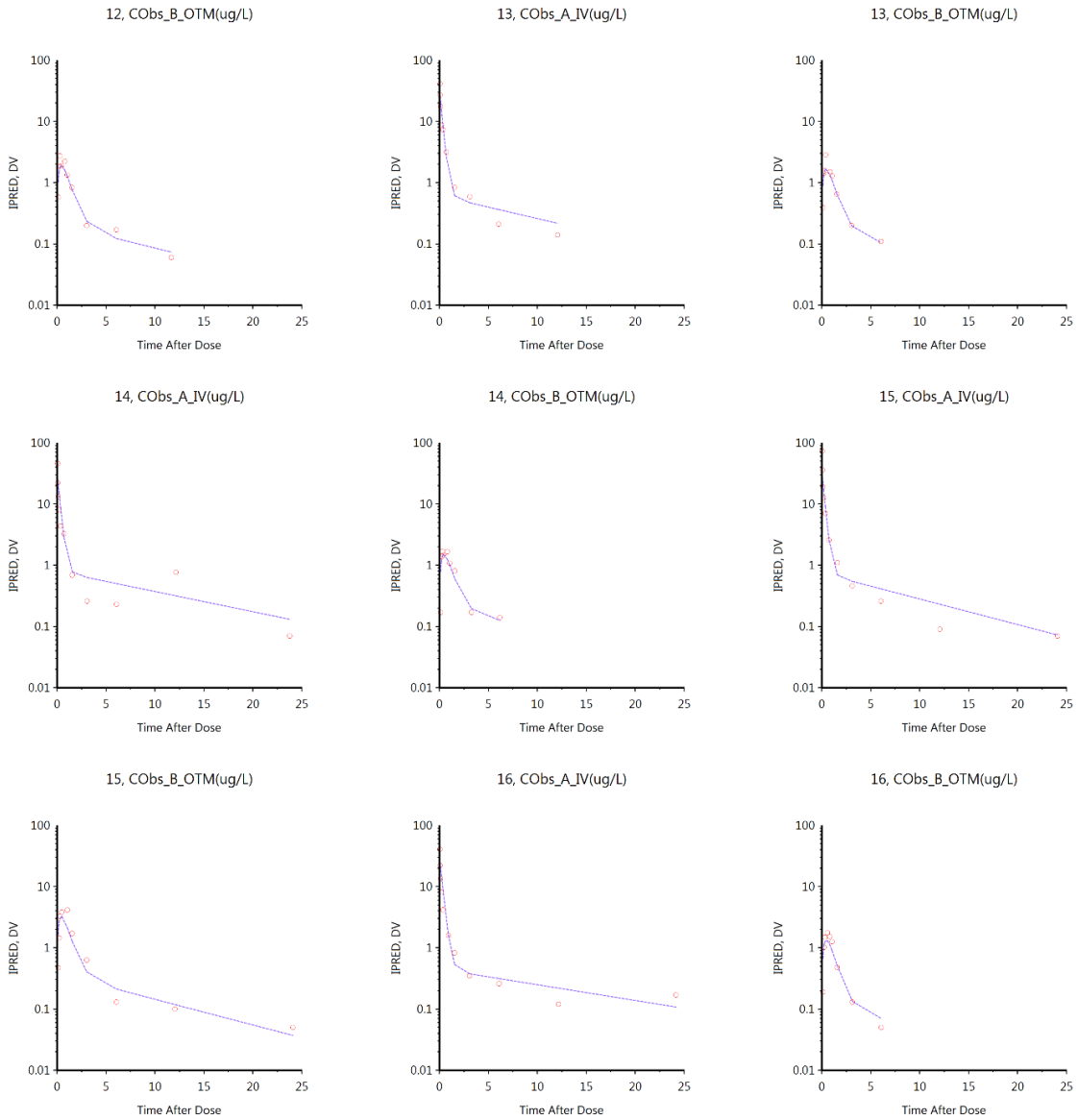


Fig suppl. 5: Invididual observed concentrations and model predictions vs time. Cats 2 to 7 has gingivostomatitis and were sparsely sampled after administration of buprenorphine 0.02 mg/kg buccally (Formulation: Buprecare, Animalcare). Cats 11 to 16 were normal cats and were densely sampled after administration of 0.02 mg/kg buprenorphine IV (CObs_A) and buccally (Cobs_B) (Formulation: Buprenex, Reckitt Beckiser Pharmaceuticals)





434

435

436

437

438

439

440

441

Conflict of interest

The authors declared no potential conflict of interest for the completion of this study.

Funding

The authors received no kind of financial support for the research, authorship or publication of this article.

References

1. Lascelles D, Capner C and Waterman-Pearson A. Current British veterinary attitudes to perioperative analgesia for cats and. *The Veterinary Record*. 1999; 145: 601-4.
2. Dohoo SE and Dohoo IR. Factors influencing the postoperative use of analgesics in dogs and cats by Canadian veterinarians. *The Canadian Veterinary Journal*. 1996; 37: 552.
3. Watson A, Nicholson A, Church D and Pearson M. Use of anti-inflammatory and analgesic drugs in dogs and cats. *Australian veterinary journal*. 1996; 74: 203-10.
4. Bortolami E and Love EJ. Practical use of opioids in cats: a state-of-the-art, evidence-based review. *Journal of feline medicine and surgery*. 2015; 17: 283-311.
5. Lutfy K and Cowan A. Buprenorphine: a unique drug with complex pharmacology. *Current neuropharmacology*. 2004; 2: 395-402.
6. Joubert K. The use of analgesic drugs by South African veterinarians: continuing education. *Journal of the South African Veterinary Association*. 2001; 72: 57-60.
7. Robertson S, Taylor P and Sear J. Systemic uptake of buprenorphine by cats after oral mucosal administration. *The veterinary record*. 2003; 152: 675-8.
8. Taylor P, Robertson S, Dixon M, et al. Morphine, pethidine and buprenorphine disposition in the cat. *Journal of veterinary pharmacology and therapeutics*. 2001; 24: 391-8.
9. Steagall P, Monteiro-Steagall B and Taylor P. A review of the studies using buprenorphine in cats. *Journal of veterinary internal medicine*. 2014; 28: 762-70.
10. Robertson S, Lascelles B, Taylor P and Sear J. PK-PD modeling of buprenorphine in cats: intravenous and oral transmucosal administration1. *Journal of veterinary pharmacology and therapeutics*. 2005; 28: 453-60.
11. Abbo LA, Ko DJC, Maxwell LK, Galinsky RE and Moody DE. Pharmacokinetics of buprenorphine following intravenous and oral transmucosal administration in dogs. *Veterinary therapeutics: research in applied veterinary medicine*. 2008.
12. Ko JC, Freeman LJ, Barletta M, et al. Efficacy of oral transmucosal and intravenous administration of buprenorphine before surgery for postoperative analgesia in dogs undergoing ovariohysterectomy. *Journal of the American Veterinary Medical Association*. 2011; 238: 318-28.

13. Giordano T, Steagall PV, Ferreira TH, et al. Postoperative analgesic effects of intravenous, intramuscular, subcutaneous or oral transmucosal buprenorphine administered to cats undergoing ovariohysterectomy. *Veterinary anaesthesia and analgesia*. 2010; 37: 357-66.
14. Santos LCP, Ludders JW, Erb HN, Basher KL, Kirch P and Gleed RD. Sedative and cardiorespiratory effects of dexmedetomidine and buprenorphine administered to cats via oral transmucosal or intramuscular routes. *Veterinary anaesthesia and analgesia*. 2010; 37: 417-24.
15. Mendelson J, Upton RA, Everhart ET, Iii PJ and Jones RT. Bioavailability of Sublingual Buprenorphine. *The Journal of Clinical Pharmacology*. 1997; 37: 31-7.
16. Hedges A, Pypendop BH, Shilo Y, Stanley SD and Ilkiw J. Impact of the blood sampling site on time–concentration drug profiles following intravenous or buccal drug administration. *Journal of veterinary pharmacology and therapeutics*. 2014; 37: 145-50.
17. Lyon KF. Gingivostomatitis. *Veterinary Clinics of North America: small animal practice*. 2005; 35: 891-911.
18. Dolieslager SMJ, Lappin DF, Bennett D, Graham L, Johnston N and Riggio MP. The influence of oral bacteria on tissue levels of Toll-like receptor and cytokine mRNAs in feline chronic gingivostomatitis and oral health. *Veterinary immunology and immunopathology*. 2013; 151: 263-74.
19. Dowers KL, Hawley JR, Brewer MM, Morris AK, Radecki SV and Lappin MR. Association of Bartonella species, feline calicivirus, and feline herpesvirus 1 infection with gingivostomatitis in cats. *Journal of feline medicine and surgery*. 2010; 12: 314-21.
20. Leal R, Gil S, Mcgahie D, et al. The Use Of Oral Recombinant Feline Interferon-omega In Naturally Feline Immunodeficiency Virus Infected Cats: New Insights Into An Alternative Immunomodulation Therapy. *Journal of Veterinary Internal Medicine*. 2014; 28: 729.
21. Healey KA, Dawson S, Burrow R, et al. Prevalence of feline chronic gingivo-stomatitis in first opinion veterinary practice. *Journal of Feline Medicine & Surgery*. 2007; 9: 373-81.
22. Brondani JT, Mama KR, Luna SP, et al. Validation of the English version of the UNESP-Botucatu multidimensional composite pain scale for assessing postoperative pain in cats. *BMC veterinary research*. 2013; 9: 143.
23. Lommer MJ. Efficacy of cyclosporine for chronic, refractory stomatitis in cats: A randomized, placebo-controlled, double-blinded clinical study. *Journal of veterinary dentistry*. 2013; 30: 8-17.
24. McKeown DA LT, Button J, et al. . A sensitive and specific liquid chromatography–tandem mass spectrometry method for the analysis of buprenorphine and norbuprenorphine in human plasma. In: Monitoring: TD, (ed.). *10th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology* Nice, France2007, p. 460-551.
25. Food U and Administration D. FDA guidance for industry: bioanalytical method validation. *US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research: Rockville, MD*. 2001.
26. Hedges A, Pypendop BH, Shilo-Benjamini Y, Stanley SD and Ilkiw J. Pharmacokinetics of buprenorphine following intravenous and buccal administration in cats, and effects on thermal threshold. *Journal of veterinary pharmacology and therapeutics*. 2014; 37: 252-9.
27. Pelligand L, Soubret A, King J, Elliott J and Mochel J. Modeling of Large Pharmacokinetic Data Using Nonlinear Mixed-Effects: A Paradigm Shift in Veterinary Pharmacology. A Case Study With Robenacoxib in Cats. *CPT: Pharmacometrics & Systems Pharmacology*. 2016; 5: 625-35.
28. Preoteasa E, Tâncu A, Iosif L, Imre MM, Murariu-Măgureanu C and Preoteasa C. Salivary changes related to systemic diseases in the edentulous patients. *Journal of medicine and life*. 2014; 7: 577.
29. Patel VF, Liu F and Brown MB. Advances in oral transmucosal drug delivery. *Journal of controlled release*. 2011; 153: 106-16.
30. Merola I and Mills DS. Systematic review of the behavioural assessment of pain in cats. *Journal of feline medicine and surgery*. 2016; 18: 60-76.
31. Ellis SL. Environmental enrichment: practical strategies for improving feline welfare. *Journal of feline medicine and surgery*. 2009; 11: 901-12.

530 32. Rodan I. Understanding feline behavior and application for appropriate handling and
531 management. *Topics in companion animal medicine*. 2010; 25: 178-88.
532 33. Southerden P. Review of feline oral disease 1. Periodontitis and chronic gingivostomatitis. *In*
533 *Practice*. 2010; 32: 2-7.
534 34. Chhabra N, Sharma P, Chhabra S and Gupta N. Efficacy of buprenorphine added to 2%
535 lignocaine plus adrenaline 1: 80,000 in providing postoperative analgesia after lower third molar
536 surgery. *International Journal of Oral and Maxillofacial Surgery*. 2016; 45: 1644-51.
537 35. Madhav NS, Shakya AK, Shakya P and Singh K. Orotransmucosal drug delivery systems: a
538 review. *Journal of controlled release*. 2009; 140: 2-11.
539 36. Bortolami E, Slingsby L and Love E. Comparison of two formulations of buprenorphine in cats
540 administered by the oral transmucosal route. *Veterinary Anaesthesia and Analgesia*. 2011; 38: 21-2.
541 37. Malinoff HL, Barkin RL and Wilson G. Sublingual buprenorphine is effective in the treatment
542 of chronic pain syndrome. *American journal of therapeutics*. 2005; 12: 379-84.
543 38. Taylor P, Steagall P, Dixon M, Ferreira T and Luna SPL. Carprofen and buprenorphine prevent
544 hyperalgesia in a model of inflammatory pain in cats. *Research in veterinary science*. 2007; 83: 369-
545 75.
546 39. Karlsson MO and Savic RM. Diagnosing model diagnostics. *Clinical pharmacology and*
547 *therapeutics*. 2007; 82: 17-20.

548