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RESEARCH PAPER

Pharmacokinetic and pharmacodynamic modelling of intravenous, intramuscular and subcutaneous buprenorphine in conscious cats

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Abstract

Objective To describe simultaneous pharmacokinetics (PK) and thermal antinociception after intravenous (IV), intramuscular (IM) and subcutaneous (SC) buprenorphine in cats.

Study design Randomized, prospective, blinded, three period crossover experiment.

Animals Six healthy adult cats weighing 4.1 ± 0.5 kg.

Methods Buprenorphine (0.02 mg kg^{-1}) was administered IV, IM or SC. Thermal threshold (TT) testing and blood collection were conducted simultaneously at baseline and at predetermined time points up to 24 hours after administration. Buprenorphine plasma concentrations were determined by liquid chromatography tandem mass spectrometry. TT was analyzed using ANOVA ($p < 0.05$). A pharmacokinetic-pharmacodynamic (PK-PD) model of the IV data was described using a model combining biophase equilibration and receptor association-dissociation kinetics.

Results TT increased above baseline from 15 to 480 minutes and at 30 and 60 minutes after IV and IM administration, respectively ($p < 0.05$). Maximum increase in TT (mean ± SD) was 9.3 ± 4.9 °C at 60 minutes (IV), 4.6 ± 2.8 °C at 45 minutes (IM) and 1.9 ± 1.9 °C at 60 minutes (SC). TT was significantly higher at 15, 60, 120 and 180 minutes, and at 15, 30, 45, 60 and 120 minutes after IV administration compared to IM and SC, respectively. IV and IM buprenorphine concentration-time data decreased curvilinearly. SC PK could not be modeled due to erratic absorption and disposition. IV buprenorphine disposition was similar to published data. The PK-PD model showed an onset delay mainly attributable to slow biophase equilibration ($t_{1/2}$ k_{e0} = 47.4 minutes) and receptor binding (k_{on} = 0.011 mL ng^{-1} $minute^{-1}$). Persistence of thermal antinociception was due to slow receptor dissociation ($t_{1/2}$ k_{off} = 18.2 minutes).

Conclusions and clinical relevance IV and IM data followed classical disposition and elimination in most cats. Plasma concentrations after IV administration were associated with antinociceptive effect in a PK-PD model including negative hysteresis. At the doses administered, the IV route should be

preferred over the IM and SC routes when buprenorphine is administered to cats.

Keywords analgesia, buprenorphine, cat, pharmacokinetics, routes of administration, thermal nociceptive threshold.

Introduction

Buprenorphine is a semi-synthetic partial mu (MOP) opioid agonist (Cowan et al. 1977) that usually has a delayed onset of action and long-lasting analgesic properties with few adverse effects (Lizasoain et al. 1991). In the UK, the drug has had market authorization for use in cats for several years. In this species, buprenorphine usually induces signs of euphoria with cats purring, rolling and kneading with their forepaws, but rarely causes side-effects such as vomiting or hyperthermia (Steagall et al. 2007). Buprenorphine has been found to produce variable analgesic effects in the cat, with some individuals appearing very comfortable and others experiencing pain or limited antinociceptive effects (Robertson et al. 2003a,b, 2005; Steagall et al. 2007, 2009a,b; Giordano et al. 2010). These conflicting results may be related to individual response to treatment, inappropriate dosing or inefficient routes of administration (Steagall et al. 2006, 2009a,b; Taylor et al. 2007; Giordano et al. 2010).

Studies in cats comparing the effects of opioids given by different routes confirm that the route of administration influences pharmacokinetics and analgesic effects (Taylor et al. 2001; Robertson et al. 2005; Giordano et al. 2010). The antinociceptive effects of opioids have been studied using a thermal threshold developed and validated for cats (Dixon et al. 2002). Subcutaneous (SC) administration of buprenorphine produced slow onset and rapid offset thermal antinociception and a limited increase in thermal nociceptive threshold (TT) in the cat (Steagall et al. 2007). In contrast, when the same drug was given by the intravenous (IV), intramuscular (IM) and oral transmucosal (OTM) routes, the increase in TT was much greater (Robertson et al. 2003a,b, 2005; Johnson et al. 2007). In cats undergoing ovariohysterectomy, SC administration of buprenorphine resulted in significantly higher pain scores and incidence of treatment failure compared with the IV and IM routes (Giordano et al. 2010). It is not possible to determine whether the differences in postoperative analgesia or antinociception in these studies were due to differences in bioavailability, distribution, metabolism and/or receptor binding, since to date there are no reports relating pharmacokinetic (PK) profile and the pharmacodynamic effect (PD) after SC or IM administration of buprenorphine to cats within the same experiment. The aims of this study in cats were to 1) describe the pharmacokinetics of buprenorphine after IV, IM and SC administration, 2) describe the time-course of thermal antinociception after IV, IM or SC buprenorphine and 3) estimate buprenorphine pharmacodynamic parameters by PK-PD modeling.

Material and methods

Animals

Six healthy adult domestic short haired cats were studied, five female and one male, ranging in weight from 3.9 to 4.8 kg (mean ± SD: 4.1 ± 0.5 kg). All cats were housed as a colony according to the Principles of the Sao Paulo State University Research Ethical Committee and were fed proprietary dry cat food *ad libitum*, supplemented with canned food once daily. Water was always freely available.

Before the study began, the cats were treated with anthelmintics, and vaccinated against *Chlamydia psittaci*, panleukopenia, calicivirus and feline rhinotracheitis. Serum biochemical and virological analyses as well as hematology were performed, and results were within reference limits for all cats. During testing, cats were housed individually in a quiet environment in adjacent cages equipped with wall mirrors, a bed, toys, food, water and a litter tray. All cats had been well handled and familiarized with the threshold testing procedure prior to the study. This study was approved by the Institutional Animal Care and Use Committee, School of Medicine, Sao Paulo State University, UNESP, Brazil (protocol number 568-2006).

At the beginning of every testing session, each cat was anesthetized in an acrylic chamber by delivering 5% isoflurane (Isothane; Baxter, Puerto Rico, USA) in oxygen (5 L minute^{-1}) until the loss of spontaneous movement. Subsequently, each cat was removed from the chamber and isoflurane was delivered via face mask until endotracheal intubation could be achieved with a cuffed tube. First, the hair of each cat was clipped on either side of the craniolateral aspect of the thorax. Thereafter, the cat was positioned in lateral recumbency and, under aseptic conditions, the jugular vein was

percutaneously catheterized with an 18-gauge 12 cm polyurethane catheter (Insyte/BD; Beckton-Dickinson, Sao Paulo, Brazil). The catheter was connected to a PRN-adaptor, sutured in place and covered with a light bandage. Cats were weighed and then allowed to recover in their cages after the discontinuation of isoflurane administration. Food but not water was withheld for 8 hours before catheter placement and was offered again 1 hour after drug administration.

Experimental protocol

Pharmacokinetic and TT studies were conducted concurrently. Once the cats had recovered from anesthesia (at least 1.5 hours after the end of isoflurane administration), baseline TT measurements were recorded and each cat received 0.02 mg kg^{-1} of buprenorphine (Temgesic, Schering Plough, Rio de Janeiro, Brazil) administered by the IV, IM or SC routes in a randomized, three period, cross-over design, with at least a 1-week interval between treatments. For IV administration, buprenorphine was given into the jugular catheter over 15 seconds. For IM and SC administration, the drug was injected into the epaxial muscles or under the skin between the shoulder blades, respectively. All treatments were administered using a 23-gauge × 2.5 cm needle. The volume of buprenorphine was made up to 0.3 mL with sterile 0.9% saline. Before and after IV administration, 3 mL of 0.9% saline were used to flush the catheter and the PRN-adaptor was changed afterwards. Treatments were administered by two observers not involved with testing.

The cats were observed for changes in behavior such as excitement, sedation or vomiting, and pupil size was evaluated subjectively at each testing point. The difference between TT before and after treatment (ΔT °C) was taken as the outcome variable for comparison between groups.

Pharmacokinetic study

Blood samples were withdrawn from the jugular catheter before and at 2, 5, 10, 15, 30, 45, 60, 120, 180, 240, 360, 480, 720 and 1440 minutes after drug dosing. The volume of blood collected was adjusted for each individual so that <10% of the cat's total blood volume was removed over the 24-hour study period (1–2 mL per sample). An equal volume of lactated Ringer's solution was injected after each sample was withdrawn. Blood was transferred to lithium heparin tubes and centrifuged ($1462 \times \boldsymbol{g}$) for 10 minutes. Plasma was separated and stored at −20 °C before buprenorphine assay was performed.

Measurements of thermal threshold

Thermal thresholds were measured by applying a ramped heat stimulus to elicit nociception (Dixon et al. 2002). A 5 g, 10 mm long, 10 mm wide and 5 mm deep, probe containing a heater and temperature sensor was held against the shaved skin of the lateral aspect of the thorax with an elasticated band and pressure bladder to ensure even contact between probe and skin (Dixon et al. 2002). The probe was connected to the control unit with light ribbon cable and skin temperature was measured. When activated, the probe heated at a rate of 0.6 °C $second^{-1}$ with a safety cut-off at 55 °C to prevent burns. At each test, the heater was activated, and then switched off as soon as the cat reacted to the stimulus by jumping, flinching, or turning toward the probe. If no reaction was seen, heating stopped automatically when the cut-off was reached. When heating was terminated at a response the probe temperature was simultaneously recorded as the TT. The cable was disconnected from the control unit between tests. The sensor was calibrated regularly before each phase of the study as previously described (Dixon et al. 2002). A single observer (PVMS) performed the TT tests and was blinded to the treatment. Prior to drug administration, four measurements were made at 15-minute intervals and their mean value taken as the control TT. For testing, care was taken to ensure that the cats were awake but not otherwise distracted; they were unrestrained and not sleeping, eating or playing. TT were measured before and 15, 30, 45, 60, 120, 180, 240, 360, 480, 720 and 1440 minutes after drug dosing.

Buprenorphine assay

A solid-phase extraction procedure, in a 96-well format, has been developed for buprenorphine analysis in feline plasma. 100 μL of sample, standard or QC was aliquoted into a borosilicate tube (20 μL of internal standard – buprenorphine-D_4 at 10 ng mL^{-1} in acetonitrile/water, 1/1, v/v). 400 μL of 4% phosphoric acid (aqueous) was added to each tube and then vortex mixed.

Extraction was carried out using Medium Cationic Exchange (MCX) stationary phase which allows the extraction of buprenorphine from other components contained in feline plasma. The plate was first washed with 1 mL of 5% ammonia in methanol and conditioned with 1 mL of methanol and two times 1 mL of 1% formic acid (aqueous) before the samples were loaded onto the plate. The plate was then washed with 1 mL of methanol followed by 1 mL of 1% aqueous formic acid. The compounds were eluted with two times 300 μL 5% ammonia in methanol into a 96-well plate. After blowing down the samples under a flow of nitrogen at 50 °C, the samples were reconstituted in 100 μL acetonitrile: 10 mmol L^{-1} ammonium formate pH 3 (10:90, v:v) and 20 μL were injected in the mass spectrometer. Extracts were chromatographically separated using an Acquity UPLC with a reverse phase BEH C18 column (100 × 2.1 mm, 1.8 μm particle size) attached to an Acquity pre-column filter. The column temperature was set at 60 °C. Separation was achieved using a slow gradient with acetonitrile and 10 mmol L^{-1} ammonium formate pH 3 in a 4-minute run time at 0.6 mL $minute^{-1}$. Ionization was performed by a TurboIonSpray at 750 °C with an IonSpray voltage of 5500 V. Acquisition was performed using a triple-Quad Mass Spectrometer API5000, in multiple reaction monitoring mode with precursor/product ion transitions of m/z 468.4 → 396.2 buprenorphine and m/z 472.4 → 400.2 for buprenorphine-D_4. Buprenorphine was quantified by linear regression with a $1/x^2$ weighting factor over the range 0.05–50 ng mL^{-1}. The lower limit of quantification (LLOQ) was 0.05 ng mL^{-1}. The intra-assay coefficients of variation (CV) were 5.4% at 0.05 ng mL^{-1}, 4.9% at 0.15 ng mL^{-1}, 2.7% at 5 ng mL^{-1} and 1.9% at 40 ng mL^{-1}. The inter-assay coefficients of variation (CV) were 12.5% at 0.15 ng mL^{-1}, 12.4% at 5 ng mL^{-1} and 9.0% at 40 ng mL^{-1}. The intra-assay relative errors were 11.0% at 0.05 ng mL^{-1}, −5.3% at 0.15 ng mL^{-1}, −7.6% at 5 ng mL^{-1} and −8.3% at 40 ng mL^{-1}. The inter-assay relative errors were 3.3% at 0.15 ng mL^{-1}, −3.4% at 5 ng mL^{-1} and −5.3% at 40 ng mL^{-1} (acceptance criteria ≤15% or ≤20% at LLOQ for all these tests). A selectivity test was performed on six different individual blank feline plasma and no interference above the limit of quantification was detected at the retention time of buprenorphine (approximately 2.5 minutes). All the samples were analyzed successfully either non-diluted or with a ten-fold dilution (CV for dilution control samples was 3.2%).

Pharmacokinetic analysis

Drug concentration-time data were analyzed for all three routes by compartmental analysis, using least-squares regression (WinNonlin version 5.2; Pharsight, CA, USA). Plasma buprenorphine concentration-time course $C_p(t)$ for each cat was best described using equations corresponding to drug disposition in a three-compartmental model (IV administration, eqn 1), or a two-compartmental model with absorption phase (IM administration, eqn 2):

$$C_p(t) = Y_1.e^{-\lambda_1.t} + Y_2.e^{-\lambda_2.t} + Y_3.e^{-\lambda_3.t} \quad (1)$$

$$C_p(t) = -(Y_4 + Y_5).e^{-k_a.t} + Y_4.e^{-\lambda_4.t} + Y_5.e^{-\lambda_5.t} \quad (2)$$

Where λ_1 to λ_5 are the slopes of the successive pharmacokinetic phases, t is the time, Y_1 to Y_5 are the intercepts on the Y axis when $C(t)$ is plotted on a semi-logarithmic scale and k_a is the first-order absorption rate constant. Data were weighted by the reciprocal of the predicted value. Goodness of fit and selection of the appropriate model were evaluated using Akaike's Information Criterion (AIC) and by visual inspection of the fitted curves and residuals.

From concentration data obtained, total area under the curve (AUC), plasma clearance (CL = dose/AUC), apparent volume of distribution during the elimination phase (V_d = CL × λ_3 for IV and V_d = CL/λ_5 for IM routes, respectively), mean residence time (MRT), apparent volume of distribution at steady state (V_{ss} = CL.MRT), and C^0 = extrapolated plasma concentration at time zero were calculated. In the SC group, pharmacokinetic analysis was not possible due to erratic absorption.

For the IM and SC administration, the maximum plasma buprenorphine concentration (C_{max}) and time of maximum drug concentration (T_{max}) were calculated. Clearance and volume of distribution indexed by F were reported for IM administration. Average bioavailability (F) was calculated using the ratio of mean AUC_{IM}/AUC_{IV} of six cats and indexing it with the ratio of average terminal elimination half-lives. Pharmacokinetic data are presented as geometric means (95% CIs) except C^0 and C_{max}, and K_a (mean ± SD), and half-lives and T_{max} which are presented as harmonic mean (±pseudo SD) estimated by the jack knife method (Lam et al. 1985).

Pharmacokinetic-pharmacodynamic analysis

Pharmacodynamic models were fitted to individual data to describe the time-course of the elevation of TT (ΔT °C) above measured baseline TT (TT_0). Models accounting for negative hysteresis were compared: simple E_{max} model with effect compartment (model 1: effect linked with concentration of the drugs at a hypothetical biophase site of action) (Shimada et al. 1996; Jensen et al. 2008), receptor association/dissociation model (model 2) (d'Hollander & Delcroix 1981) and effect compartment model combined with receptor association/dissociation model (model 3) (Shimada et al. 1996; Yassen et al. 2005). The effect compartment model combined with receptor association model (Fig. 1) best described the data as it had the lowest average AIC ($AIC_{model\ 1}$ = 43.71, $AIC_{model\ 2}$ = 50.61, $AIC_{model\ 3}$ = 43.68). Distribution of buprenorphine to the site of action (biophase) was defined by eqn 3:

$$\frac{d[C_e]}{dt} = k_{e0}.([C_p] - [C_e]) \quad (3)$$

where C_p and C_e are the plasma and effect compartment concentration of buprenorphine respectively and k_{e0}, is the first order rate constant describing the rate of change of buprenorphine concentration in the effect compartment (minute^{-1}). Buprenorphine binds the receptor at the site of action and the rate of drug-receptor binding ($d[C_eR]/dt$) depends on $[C_e]$, the free receptor concentration $[R]$ as shown in eqn 4:

$$\frac{d[C_eR]}{dt} = k_{on}.[C_e].[R] - k_{off}.[C_eR] \quad (4)$$

where k_{on} is a second-order rate constant (mL ng^{-1} minute^{-1}) governing the rate of association and k_{off} is a first order rate constant (minute^{-1}) describing the rate of dissociation of the drug-receptor complex. The total number of mu opioid receptors in the biophase $[R_{tot}]$ is the sum of the free receptors and the bound receptors as shown in eqn 5: $[R_{tot}] = [C_eR] + [R]$. Rearranging eqn 5 and replacing $[R]$ in eqn 4 yields eqn 6:

$$\frac{d[C_eR]}{dt} = k_{on}.[C_e].([R_{tot}] - [C_eR]) - k_{off}.[C_eR] \quad (6)$$

We assumed that the pharmacological effect of buprenorphine (ΔT °C) is proportional to $[C_eR]$ and that the maximal effect ΔT_{max} is obtained when $[C_eR] = [R_{tot}]$, the relationship between C_e and ΔT is as follows:

$$\frac{d\Delta T}{dt} = k_{on}.[C_e].(\Delta T_{max} - \Delta T) - k_{off}.\Delta T \quad (7)$$

Due to the safety limit of the test system, ΔT_{max} was set as (55° − TT_0). Pharmacokinetic parameters describing plasma buprenorphine disposition after intravenous administration were used as input for the PK-PD model. Equation 7 was fitted to the time course of variations of ΔT. C_e was estimated with eqn 3. Three parameters were estimated by the model: k_{e0}, k_{on}, k_{off}. K_D, the dissociation constant was defined as k_{off}/k_{on} and corresponds to the biophase concentration necessary to occupy 50% of the receptors. Average half-lives corresponding to k_{off} and k_{e0} were calculated as ln(2)/rate constant. In the IM group, PK-PD modeling was attempted but not successful due to intra- and inter subject variability. The resulting PK-PD modeling for the concentration-effect data following IV dosing are shown as geometric means (95% CIs) unless otherwise stated.

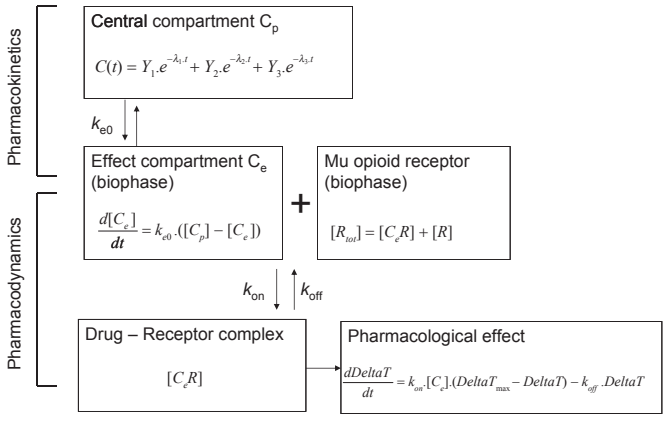

Figure 1 Pharmacokinetic-Pharmacodynamic model describing the effect of buprenorphine on thermal threshold (TT) in the cat. Negative hysteresis is due to diffusion delay in the biophase (k_{e0}) and slow binding to the opioid receptor (k_{on}).

Statistical analysis

All statistical analyses were performed using computer software (GraphPad Prism; GraphPad Software Inc., CA). Normal distribution of each measured variable (skin temperature and TT) was confirmed using the Shapiro-Wilk test. TT for each treatment were analyzed for temporal changes by means of a one-way repeated measures ANOVA, followed by the Dunnett's test when appropriate. Treatments were compared by use of a two-way ANOVA followed by a Bonferroni post-test. TT are reported as mean ± SD. A value of $p < 0.05$ was considered significant for all analysis.

Results

No adverse effects, such as vomiting, were observed after administration of buprenorphine and none of the cats was sedated after treatment. All animals ate, drank, defecated, urinated and groomed normally throughout the testing periods. Buprenorphine caused marked mydriasis in all cats independent of the route of administration. This was observed from 10 to 15 minutes after injection. Cats displayed signs of euphoria (increased purring, meowing, rubbing against handlers, especially when the cage was opened, kneading with forepaws and rolling) after all treatments.

Disposition of buprenorphine by IV, IM and SC routes

Buprenorphine plasma concentrations (ng mL^{-1}) are shown in Fig. 2. Plasma buprenorphine pharmacokinetic parameters are shown in Table 1. The IV and IM but not the SC route data were suitable for compartmental PK modeling as they followed classical disposition, absorption and elimination. Individual buprenorphine PK fitting after IV, IM and SC administration are represented in Fig. 3.

Buprenorphine plasma concentrations were measurable at all time samples in the IV and IM group, except in one cat (after IM and IV treatment) which had its last measurable concentration at 720 minutes. Following IV administration, maximum extrapolated plasma concentration (C^0) was 84.8 ± 40.6 ng mL^{-1}. Mean clearance by IV route was 7.8 (5.1–12) mL kg^{-1} $minutes^{-1}$, MRT was 336 (117–756) minutes (geometric means and 95% CI) and terminal half-life was 420 ± 382 minutes (harmonic mean ± pseudo SD).

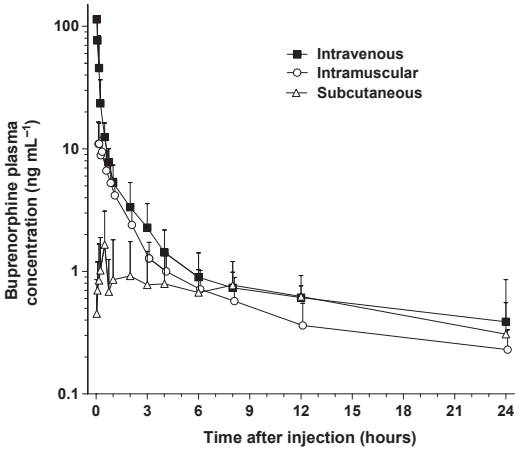

Figure 2 Plasma concentration (ng mL^{-1}, mean ± SD) following administration of 0.02 mg kg^{-1} of buprenorphine by the intravenous ($n = 6$), intramuscular ($n = 6$) or subcutaneous ($n = 5$) route in conscious cats.

Following IM administration, the maximum plasma concentration (C_{max}) was 12.1 ± 3.7 ng mL^{-1} and was observed at 5 minutes with K_a = 1.7 ± 1.3 $minute^{-1}$. Plasma clearance indexed by F was 14.2 (10.1–19.2) mL kg^{-1} $minute^{-1}$. Terminal half-life was 460 ± 285 minutes. Following IM dosing, the sample systemic bioavailability (F) was 45.7%.

In the SC group, the concentration-time data could not be modeled because of the presence of several plasma concentration peaks and the uncertainty about the slope of the elimination phase (there being an inadequate number of data sampling points to appropriately characterize the elimination phase). In one cat, plasma concentrations were still rising at the last sampling point, 1440 minutes. Another cat accidentally dislodged its jugular catheter immediately after drug administration. Some cats had early T_{max} values (15–30 minutes), but in one cat T_{max} was delayed to 720 minutes. The mean C_{max} was 1.37 (range 0.58–3.55) ng mL^{-1}. As an indication of inter-animal variability, the AUC to 6 hours post-dosing ranged between 68.1 and 707.5 ng mL^{-1} minutes.

Thermal thresholds

There were no significant changes in skin temperature in any of the treatment groups over time. Pretreatment TT (mean ± SD) was 40.7 ± 1.9 °C, 41.4 ± 1.7 °C and 41.9 ± 2.0 °C in the IV, IM and SC group, respectively ($p > 0.05$). After IV administration, TT was significantly higher than baseline

Table 1 Pharmacokinetic (PK) analysis of plasma concentrations following intravenous (IV), intramuscular (IM) or subcutaneous (SC) administration of 0.02 mg kg^{-1} of buprenorphine in conscious cats. All pharmacokinetic parameters are presented as geometric means (95% CIs) except C^0 and C_{max}, and K_a (mean ± SD), and half-lives and T_{max} which are presented as harmonic mean ± pseudo SD estimated by the jackknife method. C^0 is the extrapolated concentration at time 0 for IV administration and C_{max} the peak plasma concentration after IM or SC dosing. K_a is the absorption rate constant and K_{10} $t_{1/2}$ HL is the half-live relative to the elimination rate constant (K_{10}), Terminal $t_{1/2}$ HL is the half-life relative to the terminal phase

PK variables	IV (*n* = 6)	IM (*n* = 6)	SC (*n* = 5)
T_{max} (minutes)	–	2.7 ± 1.6	(range 15–720)
C^0 or C_{max} (ng mL^{-1})	84.8 ± 40.6	12.1 ± 3.7	1.37 (range 0.58–3.55)
K_a ($minute^{-1}$)		1.7 ± 1.3	NC
K_{10} $t_{1/2}$ HL (minutes)	22.8 (16.1–32.1)	75.8 (46.2–120.5)	NC
AUC (h × ng mL^{-1})	42.7 (16.7–79.8)	23.5 (17.8–30.6)	NC
F (%)		45.7	NC
MRT (minutes)	336.2 (117.2–756.4)		NC
Terminal $t_{1/2}$ HL (minutes)	420 ± 382	460 ± 285	NC
CL or CL/*F* (mL kg^{-1} $minute^{-1}$)*	7.8 (5.1–12.00)	14.2 (10.1–19.2)	NC
V_d or V_d/F (L kg^{-1})*	2.92 (1.69 – 5.05)	10.3 (5.2–20.5)	NC
V_{ss} (L kg^{-1})	2.62 (0.45–6.66)	NC	NC

The bioavailability (*F*) was calculated from AUC ratios.

AUC, area under the plasma concentration-time curve; MRT; mean residence time; CL or CL/*F*, systemic clearance or apparent clearance; apparent volume of distribution at pseudo equilibrium during the elimination phase (V_d or V_d/F); V_{ss}, apparent volume of distribution at steady state; NC, non calculable.

*Data for IM are shown as clearance and apparent volume of distribution not corrected for bioavailability.

from 15 to 480 minutes and after IM administration at 30 and 60 minutes (Fig. 4). TT did not increase significantly above baseline after SC administration (Fig. 4).

Maximum increase in TT above baseline was 9.3 ± 4.9 °C at 60 minutes ($p < 0.05$), 4.6 ± 2.8 °C at 45 minutes ($p > 0.05$) and 1.9 ± 1.9 °C at 60 minutes ($p > 0.05$) after IV, IM and SC administration, respectively. The TT cut-off was reached in two cats after IV administration (3 time points in total). TT were significantly higher at 15, 60, 120 and 180 minutes and at 15, 30, 45, 60 and 120 minutes in the IV group compared with the IM and SC groups, respectively. TT were not significantly different when the IM and SC groups were compared (Fig. 4).

Pharmacokinetic-pharmacodynamic relationships

The plasma concentration and effect data from each cat were plotted to demonstrate negative hysteresis (Fig. 5). The PK-PD relationship was examined following dosing by the IV route and was best described by a combined effect compartment/receptor association-dissociation model and buprenorphine estimated parameters after IV dosing are reported in Table 2. The effect compartment rate constant k_{e0} was 0.015 $minute^{-1}$ (0.0043–0.0502) yielding an average equilibration half-life of 47.4 minutes (13.8–160.2) between plasma and biophase. Receptor association (k_{on}) and dissociation rate constants (k_{off}) were 0.011 mL ng^{-1} $minute^{-1}$ and 0.038 $minute^{-1}$, respectively, yielding an average receptor dissociation half-life of 18.2 minutes. Harmonic mean of the dissociation constant (K_D) was 2.7 (1.48–28.94) ng mL^{-1}.

Discussion

This study showed that the route of administration will influence the onset, duration, and magnitude of analgesia when buprenorphine is administered to cats. A similar effect of route of administration was seen in a previous study using the same TT device, where slow onset and rapid offset were observed after SC buprenorphine (Steagall et al. 2006) while other routes of administration provided much greater antinociception (Robertson et al. 2003a,b, 2005; Steagall et al. 2009a). In experimental settings, 0.02 mg kg^{-1} of buprenorphine administered by the SC route produced a maximal mean TT of 44.8 °C (Steagall et al. 2007) whereas when the same drug was given by the IV, IM and OTM routes, TT increased up to 53.6, 51 and 51.4 °C,

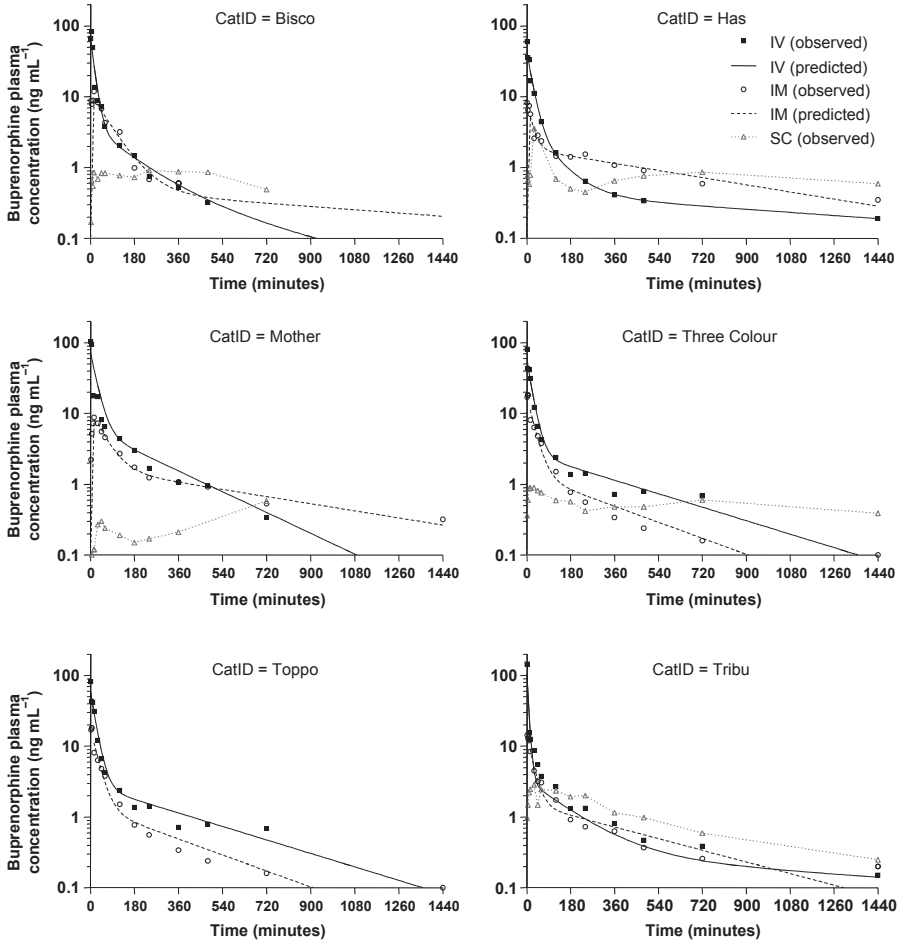

Figure 3 Individual observed plasma concentrations and fitted PK modeling after intravenous, intramuscular and subcutaneous injection of 0.02 mg kg^{-1} of buprenorphine.

respectively (Robertson et al. 2003a,b, 2005). In the present study, SC buprenorphine did not induce significant antinociception and produced a maximal mean TT of 43.8 °C. These findings are in agreement with clinical data where, in cats undergoing ovariohysterectomy, SC and OTM administration of buprenorphine was associated with significantly higher pain scores than those receiving the drug by the IV and IM routes (Giordano et al. 2010). Furthermore, in that study, there was a significantly higher incidence of treatment failure in the SC group (52%) when compared with the IV (24%) and IM (16%) groups.

The current PK analysis supports these observations. Buprenorphine uptake and plasma concentrations were very low after SC administration in comparison to IV and IM administration. The resulting low concentration gradient does not allow the drug to reach effective concentrations in the biophase, presumably leading to occupation of fewer opioid receptors in the CNS. After IV and IM dosing, higher plasma buprenorphine concentrations allow drug to transfer down the concentration gradient into the central nervous system to yield concentrations greater than K_D leading to the occupation of more opioid receptors and in turn a greater antinociceptive effect.

Higher peak plasma concentrations of buprenorphine were detected after application of a transdermal matrix patch (5.37–13.7 ng mL^{-1}) (Murrell et al. 2007) than after SC administration in the present study. However, there were no significant changes in TT over time nor a strong relationship between buprenorphine plasma concentration and changes in TT in either study, suggesting that a large initial concentration gradient of buprenorphine may be needed in order to drive the drug into the biophase. However, buprenorphine plasma

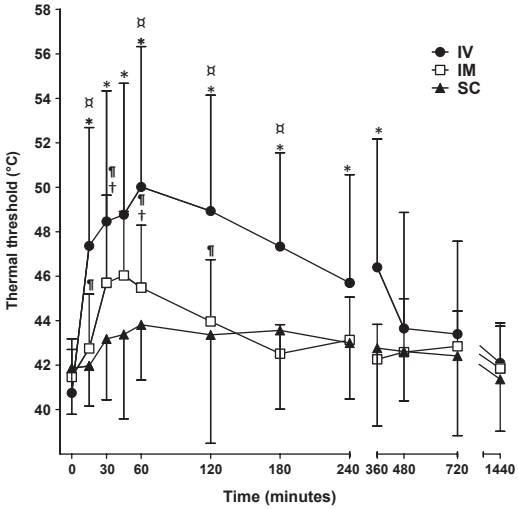

Figure 4 Thermal thresholds (TT) (°C, mean ± SD) following administration of 0.02 mg kg^{-1} of buprenorphine by the intravenous (n = 6), intramuscular (n = 6) or subcutaneous (n = 6) route in conscious cats. *TT increased above baseline from 15 to 480 minutes in the IV group ($p < 0.05$). †TT increased above baseline from 30 and 60 minutes in the IM group ($p < 0.05$). ¤TT were significantly higher at 15, 60, 120 and 180 minutes in the IV group compared with the IM group ($p < 0.05$). ¶TT were significantly higher at 15, 30, 45, 60 and 120 minutes in the IV group compared with the SC groups ($p < 0.05$). TT were not significantly different when the IM and SC groups were compared ($p > 0.05$).

concentrations were sustained at a low level after SC administration and multiple plasma peak concentrations were observed (Figs 2 and 3). In one cat, plasma concentration continued to rise in the SC group at the 24 hour sampling point. This effect could be explained by delayed absorption (''flip flop'' kinetics usually seen with slow absorption of depot formulations), absorption from multiple sites or entero-hepatic recycling, suggesting a slow and unpredictable absorption and disposition from the SC route (Toutain & Bousquet-Melou 2004).

Although an antinociceptive effect was not detected after SC administration of buprenorphine, the behavioral changes following administration of buprenorphine were still observed in both this and the previous study (Steagall et al. 2006) examining the effects of this route of administration. Clinicians often use the SC route of administration in dogs and cats as the injection is less painful and stressful compared with the IM route (Gurney et al. 2009). However, although the behavioral effects, including sedation, may be similar after IM or SC drug administration, analgesic effects were much reduced when buprenorphine was administered SC to cats. There is a tendency to associate changes in behavior with good analgesic effects after buprenorphine administration in cats. These changes probably occur at concentrations of buprenorphine that are lower than those required to produce analgesia (Murrell et al. 2007) and it is possible that the K_D for behavioral changes is lower than the K_D for thermal antinocicepion estimated in the present study. Our results further emphasize that the SC route, at the doses studied here, is unsuitable for producing analgesia in cats in a clinical setting. Similar observations have been reported after SC administration of hydromorphone which also resulted in short duration antinociception and least effect when compared with IV and IM routes (Lascelles & Robertson 2004; Wegner & Robertson 2007; Robertson et al. 2009).

Maximum increase in TT above baseline was observed at 60 minutes after IV injection. Previous attempts at PK-PD modeling in the cat have shown a poor correlation between antinociceptive efficacy and the plasma concentration of buprenorphine due to negative hysteresis (Robertson et al. 2005; Murrell et al. 2007) and it has been difficult to predict how small differences in the PK results might

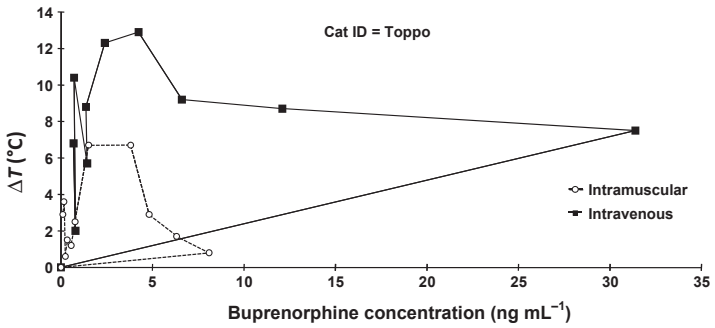

Figure 5 Concentration-effect (ΔT °C) plot for a representative cat demonstrating negative hysteresis following IV and IM injection of 0.02 mg kg^{-1} of buprenorphine.

Table 2 Estimated pharmacokinetic-pharmacodynamic (PK-PD) parameters describing antinociceptive effect on thermal threshold (TT) following administration of 0.02 mg kg^{-1} of buprenorphine by the intravenous route in six conscious cats

PD parameters	Values
Parameters estimated from PK-PD model	
k_{on} (mL ng^{-1} $minute^{-1}$)	0.011 (0.00022–0.0266)
k_{off} ($minute^{-1}$)	0.038 (0.0118–0.0537)
k_{e0} ($minute^{-1}$)	0.015 (0.0043–0.0502)
Parameters calculated from estimated parameters above	
K_D (ng mL^{-1})	2.7 (1.48–28.94)
$t_{1/2}$ k_{e0} HL (minutes)	47.4 (13.8–160.2)
$t_{1/2}$ k_{off} HL (minutes)	18.2 (6.2–107.4)

PD parameters are presented as mean (range), except for half-lives and *K*d which are reported as harmonic means (range). A combined effect compartment/receptor association-dissociation model was used and the estimations of three PD parameters: k_{e0}, the rate constant describing the rate of change of buprenorphine concentration in the effect compartment ($minute^{-1}$), k_{on} the rate constant (mL ng^{-1} $minute^{-1}$) governing the rate of receptor association and k_{off} the rate constant ($minute^{-1}$) describing the rate of dissociation of the drug-receptor complex. The dissociation constant (K_D) was calculated as k_{off}/k_{on}.

actually affect changes in TT or analgesia. In the study by Taylor et al. (2001), TT measurements were not conducted simultaneously and hence the antinociceptive effects cannot be compared. The binding of buprenorphine to receptors is known to be slow and incomplete (Cowan et al. 1977; Lizasoain et al. 1991; Yassen et al. 2005) such that the antinociceptive changes do not parallel the plasma concentration of the drug (Murrell et al. 2007). Data presented in this paper demonstrate negative hysteresis and a considerable delay between peak plasma concentration and dynamic effect (Fig. 5) associated with a much greater magnitude of antinociception and prolonged duration of action when compared with other routes. Mechanism-based PK-PD modeling, as carried out in this study, supports the fact that negative hysteresis is mainly due to diffusion of drug in the effect compartment (K_{e0} half-life of 47.4 minutes) but also due to slow association with the receptor. At the same doses used here (0.02 mg kg^{-1}), previous studies have demonstrated rapid onset of action after IV administration (Steagall et al. 2009a). Similar peak plasma concentrations have been reported in cats when 0.02 mg kg^{-1} of buprenorphine was given intravenously (Robertson et al. 2005). In the latter study, TT changes were significantly increased up to 360 minutes when compared to baseline (Robertson et al. 2005) whereas changes in TT occurred up to 480 minutes in the present study. Slow dissociation from the receptor (k_{off} half-life of 18.2 minutes) accounts for persistence of the antinociception when plasma concentrations have returned to levels below K_D. Estimates for pharmacodynamic variables compared well with results obtained in other species. In rats, for which antinociception was tested by tail-flick latency, half-life of biophase equilibration (K_{e0} half-life) was 29 minutes and half-life for receptor dissociation (k_{off} half-life) was 9 minutes (Yassen et al. 2005). *In vivo* estimation of K_D was 3.2 ng mL^{-1} in these rats which is close to our *in vivo* estimation in cats. Pharmacodynamic variables were of the same order in human volunteers subjected to a model of acute pain induced by electrical current, as buprenorphine k_{off} half-life was 8.8 minutes and K_D was 1.24 ng mL^{-1}, but the biophase equilibration was longer than in cats and rats (K_{e0} half-life of 155 minutes) (Yassen et al. 2006).

The dose of buprenorphine used here was based on previous studies where this opioid produced dose-related antinociceptive effects in cats, with doses >0.01 mg kg^{-1} having a more pronounced antinociceptive effect (Steagall et al. 2009a; Slingsby et al. 2010).

After IV administration of buprenorphine, the PK analysis and disposition data followed classical disposition, absorption and elimination and data are comparable with those reported by Taylor et al. (2001) in six conscious cats that had received 0.01 mg kg^{-1} of buprenorphine. In this earlier study, the mean terminal half-life, mean residence time (MRT), clearance (Cl), and apparent volume of distribution at steady state (V_{ss}) were reported as 416 minutes, 418 minutes, 16.7 mL kg^{-1} $minute^{-1}$, and 7.1 L kg^{-1}, respectively; whereas in our present study, the geometric mean values for these variables were 560.4 minutes, 336.2 minutes, 7.8 mL kg^{-1} $minute^{-1}$, and 2.62 L kg^{-1}, respectively. Differences in PK data observed in these studies are probably due to both variability among individuals and the different analytical technique used to measure plasma buprenorphine. Previous investigations have used ^{125}Iodine-labelled radioimunoassay (RIA) (Taylor et al. 2001; Robertson et al. 2005; Murrell et al. 2007) or enzyme linked immunoassay (ELISA) (Duke-Novakovski et al. 2011) whereas the present study used LC-MS/MS. LC-MS/MS was able to detect lower plasma

concentrations of buprenorphine but it is unknown whether such low concentrations are of clinical relevance or play any role in analgesic efficacy. Both the RIA and ELISA assays cross-react with buprenorphine-3-glucuronide or other opioids, respectively. However, there is no evidence that significant plasma concentrations of drug glucuronides are produced in healthy cats (Taylor et al. 2001); the importance of cross-reactivity with RIA may be limited. Nevertheless, the ELISA assay detects the presence of cross-reactivity with unknown compounds (background concentration) even in cat blank plasma and could limit its application (Duke-Novakovski et al. 2011).

Administration of buprenorphine by the IM route induced rapid offset (at 30 and 60 minutes) of thermal antinociception in our study. This finding was surprising since IM administration of doses of 0.01 or 0.02 mg kg^{-1} buprenorphine produced significant and long lasting increases in TT in cats in previous studies (Robertson et al. 2003b; Johnson et al. 2007). The PK data in the present study for IM dosing are similar to results reported by Taylor et al. (2001). In our study, absorption was very rapid (K_a = 1.7 $minute^{-1}$) but geometric mean terminal half-life was 526 minutes which is similar to the one observed after IV administration. Due to the rapid offset, PK-PD modeling of IM data was unsuccessful when possible solutions for PD variables were searched within a range of values physiologically possible or previously described in the literature. The IM administration of buprenorphine has produced effective analgesia in cats undergoing ovariohysterectomy (Steagall et al. 2009a; Giordano et al. 2010) and the limited average increase in TT observed in this study could be a result of individual variation in PK and PD among cats with some responding very little and others exhibiting a greater response (Table 2, Fig. 6). According to the literature, buprenorphine appears to have a variable analgesic effect, with some cats appearing very comfortable and others experiencing pain, in both clinical and experimental settings (Gassel et al. 2005; Steagall et al. 2006, 2007, 2009a,b; Giordano et al. 2010). These studies indicate that when buprenorphine is given as a sole analgesic agent, it may not be sufficient for treatment of acute postoperative pain in some cats. This may be a result of individual response to opioids in cats (Taylor et al. 2007) and highlights the importance of assessing each patient for evidence of pain.

In the IM group, changes in TT did not differ significantly from the SC group, even though the maximum increase in TT after IM (4.6 ± 2.8 °C) was greater than after SC (1.9 ± 1.9 °C) treatment. This could be explained by the limited ability to detect statistical differences between groups. There was considerable variation in TT after treatment which was probably associated with treatment, since baseline thresholds were not significantly different among groups. Individual variation in response after analgesic treatment is likely to be caused by genetic variability and could be the main reason for sensitivity to analgesics in cats (Johnson et al. 2007; Taylor et al. 2007). This high individual variability generated large standard deviations, especially when a small group of six cats was used and no control group with saline was included. The magnitude of any change resulted in no or only small significant differences between groups or with time using ANOVA, even when the difference between TT before and after treatment was used for intergroup comparison. This has also been demonstrated in previous studies using the same methodology in cats (Robertson et al. 2005; Johnson et al. 2007; Steagall et al. 2009a) and might underlie the few significant changes in TT in the IM group. This lack of any significant antinociceptive effect may indicate an inadequate power in the present study due to the small number of animals in each dosing regimen. In a previous report, which included 5/6 cats used in the present study, TT did not change significantly after saline injection and for this reason a control group was not included in the present study as this would have caused unnecessary pain (Steagall et al. 2007).

There are a number of limitations in the design and data handling in this study. The same catheter was used for drug administration and IV sampling. Even though the PRN was changed, buprenorphine may have been adsorbed onto the catheter material and subsequently picked up by the sensitive LC-MS/MS assay. This would lead to artificially high initial plasma drug concentrations after IV administration. However, the peak concentrations compare well with previous reports (Taylor et al. 2001; Robertson et al. 2005). An alternative approach would have been use of either a double lumen jugular catheter or a separate catheter in a cephalic or saphenous vein. The 'cut-off' at 55 °C was essential for prevention of skin burns. Unfortunately this caps the peak threshold data, and, by masking the potential maximum effect inevitably affects estimation of the maximal TT

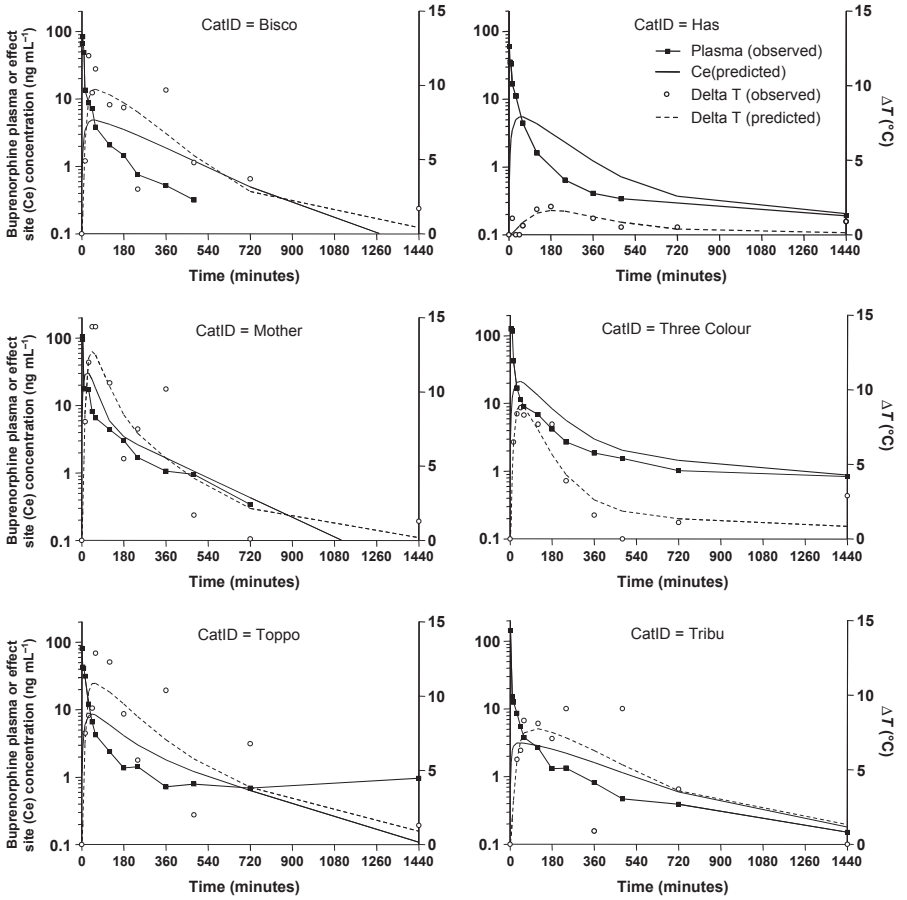

Figure 6 Individual observed plasma buprenorphine concentrations-time profile, predicted effect-compartment buprenorphine concentrations and observed and fitted ΔT °C after intravenous administration of 0.02 mg kg^{-1} of buprenorphine in six cats.

increase, making the interpretation of the PK/PD relationship more challenging. Although inclusion of a range of doses might help to elucidate the relationship further, this remains a consistent disadvantage of the thermal model.

Conclusion

The PK disposition data after IV and IM administration of buprenorphine followed classical disposition, absorption and elimination in most of the cats. IV administration of buprenorphine produced more rapid onset, greater magnitude and longer lasting antinociception when compared with the IM and SC routes. Plasma concentrations after SC administration were well below K_D, accounting for the poor effect associated with this route.

At the doses reported, the IV route should be preferred over the IM and SC routes when buprenorphine is used for clinical pain relief. This study backs up previous reports showing that SC buprenorphine provides inadequate analgesia in cats. Due to the variable individual responses, buprenorphine administration should be tailored to individual needs and not by a predetermined regimen. Since the sedation and euphoric behavior may not be related to buprenorphine's analgesic effect it is particularly important that pain itself, not some surrogate measure, is assessed during analgesic treatment.

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