Dosing protocols to improve the efficacy of butorphanol in healthy dogs

by

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Abstract

Opioid analgesics are important for perioperative pain management protocols due to their high analgesic efficacy; however, some opioids, such as butorphanol, have been reported to have a short duration of central opioid effect. The objective of this study was to compare the pharmacokinetics and effects of butorphanol in dogs after various routes of administration and to investigate strategies to prolong the duration of activity of butorphanol. Twelve Beagles (6 male, 6 female) were enrolled. Six dogs were randomly allocated to each butorphanol treatment protocol: IV (0.4 mg/kg), IV loading dose (0.2 mg/kg) with IV constant rate infusion (CRI) (0.2 mg/kg/hr for 8 hours), subcutaneous (SC) (0.4 mg/kg), SC (0.8 mg/kg) with an equal volume sodium bicarbonate (SC-bicarbonate) and IV after administration of cytochrome P450 (CYP) inhibitors. We hypothesized that; (1) administration of butorphanol as a CRI would produce a longer duration of central opioid effects and more consistent plasma concentrations than an IV bolus administration, (2) SC administration of butorphanol-bicarbonate suspension would produce a longer duration of opioid effects and more detectable plasma concentrations than SC administration of butorphanol alone, and (3) administration of CYP inhibitors prior to IV bolus administration would prolong duration of opioid effects and butorphanol plasma concentrations compared to IV bolus alone. Hypothermia, an opioid effect paralleling antinociception in dogs, and sedation were measured. Pharmacokinetics and purported CYP inhibitor effects on butorphanol pharmacokinetics were determined. Rectal temperatures were significantly lower than baseline from 1.5-4 hr (IV), 1-5 hr (CRI) and 2-7 hr (SC-bicarbonate), but not after SC. All treatments resulted in sedation. The elimination half-life of butorphanol was approximately 1.5 hr. Butorphanol via the SC-bicarbonate route had a lower bioavailability (61%) relative to SC with no sustained release observed. The mean steady state plasma concentration for CRI was

43.1 ng/mL. Purported CYP inhibitors had minor effects on butorphanol pharmacokinetics. Further studies are required to determine antinociception and clinical analgesia of these protocols.

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Acknowledgements

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Introduction

Butorphanol interacts with the μ-opiate receptor as a partial agonist and the kappa-opiate receptor as an agonist and is a commonly used analgesic and anesthetic adjunct in veterinary medicine (Garner et al., 1997). It produces typical opioid effects in dogs including analgesia and sedation, with minimal cardiopulmonary depression at clinically relevant dosages (Houghton, et. al., 1991; Sawyer, et. al. 1991; Trim, 1993). Butorphanol provides mild to moderate analgesic effects at specific dosages with minimal adverse effects and has lower abuse potential compared to pure μ-agonist opioids such as morphine and hydromorphone (Houghton et al., 1991; Sawyer er al 1991; Wegner et al., 2008). Since butorphanol is only FDA-approved as an antitussive in dogs, it is used in an extra-label manner for its analgesic activity.

Currently, canine pharmacokinetic data for butorphanol are limited to subcutaneous (SC), intramuscular (IM) and epidural routes of administration (Pfeffer et al., 1980; Troncy et al, 1996). In a previously published study using six healthy Beagles, the terminal half-life after SC and IM administration was 1.5-1.7 hr at 0.25mg/kg with rapid absorption and a maximum concentration ($C_{MAX)}$ range of 25-33 ng/mL (Pfeffer et al., 1980). To date, no pharmacokinetic data have been published in the literature for intravenous (IV), oral (PO) or constant rate infusion (CRI) administration of butorphanol in dogs.

Previously published pharmacodynamic studies indicate that butorphanol causes dose-dependent opioid effects such as sedation, miosis, hypothermia and antinociception in dogs. Butorphanol produced dose-related miosis after SC administration in dogs with a maximum effect at 0.25 mg/kg (Pircio et al., 1976). Butorphanol (0.4 mg/kg IV) was observed to produce equianalgesic peak effects and duration compared to hydromorphone (0.1 mg/kg IV) in an objective model of thermal antinociception in dogs (Wegner et al., 2008). Butorphanol, either at

0.4 mg/kg IV or 0.8 mg/kg SC, produced significant antinociception with a canine colonic distension model (Houghton, et. al., 1991; Sawyer, et. al. 1991). Other studies have demonstrated that butorphanol has minimal effects on various cardiovascular and respiratory factors when administered alone (IV) or in combination with a commonly used sedative tranquilizer (Trim, 1983; Houghton, et. al., 1991; Sawyer, et. al. 1991; Gomes et al., 2018).

Rectal temperature is a parameter used as a non-invasive surrogate marker for central opioid effects that parallels analgesic/antinociceptive dose-response effects (Vaupel & Jasinski, 1997; KuKanich et al., 2019, 2020, 2021). Butorphanol has been reported to decrease rectal temperature in dogs, but the duration of effect was not determined (Gomes et al., 2018). A decrease in rectal temperature is likely caused by a central opioid effect on the thermoregulatory center of the hypothalamus (Adler et al., 1988).

The SC and IM administration of butorphanol has been reported to cause pain upon injection potentially due to its acidic formulation (pH 3.5-5.0) (Zoetis Manufacturing, Research Spain SL, Girona, Spain). A strategy to decrease pain associated with the injection is to neutralize the pH by adding sodium bicarbonate. Previous studies have demonstrated sodium bicarbonate and butorphanol could be mixed in the same syringe (KuKanich, unpublished data). Upon mixture of butorphanol and sodium bicarbonate, a visible precipitation occurs within approximately 30 minutes creating a poorly water-soluble suspension. A neutralized butorphanol-bicarbonate suspension could reduce painful reaction upon administration as well as slow absorption and prolong opioid effects but studies are needed prior to recommending clinical use.

Butorphanol has been reported to have a rapid elimination half-life and clearance resulting in a short duration of effect (Wegner et al., 2008; Pfeffer et al., 1980). Butorphanol is

metabolized to the inactive metabolite hydroxybutorphanol, most likely through cytochrome P450 (CYP) metabolizing enzymes (Gaver et al., 1981). However, the specific CYP isoforms involved in butorphanol metabolism have not been identified. One strategy to increase plasma concentrations and duration of butorphanol's effects is to investigate administration of CYP inhibitors prior to administration of butorphanol. Previous studies have reported increased maximum plasma concentrations and significantly prolonged opioid effects after oral administration of methadone with CYP inhibitors compared to methadone alone (KuKanich et al., 2019, 2020).

The purpose of this study was to determine the pharmacokinetics and pharmacodynamics of butorphanol following different routes of administration and in combination with other commercially available drugs to healthy dogs to identify dosing protocols that may provide sustained antinociceptive effects in dogs. We hypothesized the following; (1) administering butorphanol at 0.4 mg/kg IV or SC would result in a duration of opioid effects for 1-2 hours, while a 0.2 mg/kg loading dose followed by a 0.2 mg/kg/hr CRI would provide a prolonged and consistent opioid effect throughout the 8-hour infusion, (2) when butorphanol injection is mixed with commercially available sodium bicarbonate injection resulting in a butorphanol precipitate and administered subcutaneously (SC-bicarbonate), a prolonged opioid effect would be produced as compared to butorphanol administered SC alone due to slow absorption of the poorly water-soluble precipitate resulting in flip-flop pharmacokinetics, and (3) administration of CYP inhibitors prior to administration of butorphanol would result in significantly decreased clearance, prolonged half-life and prolonged duration of opioid effects due to inhibition of CYP-mediated butorphanol metabolism. The long-term goal of this study is to develop protocols for

butorphanol with potential clinical application while using commercially available formulations to provide extended opioid effects including adequate analgesia.

Methodology

Animals

All study protocols were approved by the Kansas State University Institutional Animal Care and Use Committee (protocol no. 4553). Twelve healthy purpose bred Beagles (Ridglan Farms Inc, Mount Horeb, WI, USA) (6 neutered males; 6 spayed females) aged 1 year and weighing 5.4 - 9.8 kg were included in the study (Figure 1). The study was conducted in two phases in which all 12 dogs were used for phase 1, and 6 dogs from phase 1 were used for phase 2. Each dog was randomly assigned to treatment groups by choosing one piece of paper with the dog's name from a box, and a separate piece of paper with a number that determined treatment assignment from a different box. All dogs were deemed healthy prior to inclusion in the study. Health status was determined on the basis of history and the results of physical examination, complete blood count, and serum biochemical analysis. Dogs were routinely housed in compatible pairs or groups in indoor runs (10 x 10 feet) with outdoor access when the weather was appropriate. During the study period, dogs were housed individually in runs (5x7 feet) that included elevated dog beds. Dogs were returned to their routine housing at the end of the study. Dogs were housed in 2x2 - 3x5 feet stainless steel kennels during sample collection. Prior to any treatment, dogs were fasted for at least 12 hours and water was offered after the 8-hour time point, while food was offered after the 12-hour time point.

Drug Administration.

An incomplete block design with n=6 dogs per treatment with no more than 3 treatments per dog was used (Figure 1). There was at least a 10-day washout period between treatments. Phase 1 consisted of four different treatment groups (n = 6 dogs per group). Group 1 dogs were

administered butorphanol (Zoetis, Research Spain SL, Girona, Spain) at 0.4 mg/kg IV once (IV bolus) as one treatment and an IV loading dose of 0.2 mg/kg followed by a CRI at 0.2 mg/kg/hr for 8 hours equaling a total dose of 1.8 mg/kg over 8 hours (IV CRI) as a different treatment in a randomized, complete block crossover. Prior to CRI administration a jugular catheter (19gauge, 1") was placed aseptically for drug infusion and was removed at the end of the infusion. The infusion was administered using a calibrated fluid pump (Practivet, Tempe AZ, USA) with a burette (JorVet, J-468B, Jorgenson Laboratories Inc, Loveland CO, USA) attached to an IV infusion set (Practivet). Group 2 dogs were administered butorphanol 0.4 mg/kg SC once (SC) as one treatment and butorphanol at 0.8 mg/kg mixed with equal volume of 8.4% sodium bicarbonate (Vet One, Nova-Tech, Grand Island, NE, USA) administered subcutaneously once (SC-bicarbonate) as a different treatment in a randomized, complete block crossover. The alkaline bicarbonate solution caused precipitation of butorphanol within 30 minutes and resulted in a poorly water-soluble drug suspension.

The second phase of the study was conducted at least 4 weeks after Phase 1. Six dogs that had received butorphanol as an IV bolus from Phase 1 were included. In this phase, each dog received the same treatment. The following purported CYP inhibitors (targeted doses) were all administered concurrently 1.5-2 hours prior to butorphanol 0.4 mg/kg IV: chloramphenicol 50 mg/kg PO (CYP-2B11), cimetidine 50 mg/kg PO(CYP isoform unknown), citalopram 2 mg/kg PO (CYP-2D15) and ketoconazole 20 mg/kg PO (CYP-3A12/26) (IV inhibitors) (Lindstrom et al.,1987, KuKanich et al., 2015, Perez-Jimenez et al, 2019).

Sample Collection and Processing

Approximately 24 hours prior to butorphanol administration, an aseptic jugular catheter was placed (19-gauge x 8-12") after mild sedation using acepromazine (Vet One, Nova-Tech) 0.04 mg/kg SC and hydromorphone (West-Ward, Eatontown, NJ, USA) 0.05 mg/kg SC. The area over the jugular vein was clipped and cleaned with chlorhexidine scrub and isopropyl alcohol. A second jugular catheter was placed in the IV CRI group for drug administration. The catheters in the IV CRI group were marked “sample only” and “infusion only.” The jugular catheters were bandaged throughout the study and removed after the last blood sample was obtained for each treatment. If blood could not be collected from the jugular catheter, blood was obtained with phlebotomy using a 21-gauge x 1" needle from the cephalic vein.

Three mL blood samples were collected for each treatment group at predetermined time points (Table 1). Blood was placed in heparin tubes then stored on ice until centrifugation. The blood was centrifuged (3,000 x g for 15 minutes) within 2 hours after collection and the plasma was collected and stored at -80 C until analysis.

Pharmacodynamic Assessment

Dogs were evaluated for rectal temperature using a single digital thermometer and for sedation using a previously published sedation scale (Appendix A) at predetermined time points (Table 1) (KuKanich et al., 2021; Martinez et al., 2014). Sedation score was assessed by the same group of observers in which a consensus was made for each sedation score at the predetermined timepoint.

Plasma Butorphanol Concentrations

Plasma butorphanol concentrations were determined by liquid chromatography with triple quadrupole mass spectrometry (Acquity UPLC, TQD, Waters Corporation, Milford, MA, USA) based on a previous method (Paine, et. al., 2020) with some modifications. Canine plasma standards, canine plasma quality controls and incurred plasma samples were extracted and analyzed as previously described. Plasma, 200 µL, was added to 200 µL of 2% ammonium hydroxide with 100 ng/mL medetomidine (internal standard) and mixed by aspiration with the pipette. Microelution plates (Oasis HLB µElution plates, Waters Corporation) were conditioned with 200 µL of methanol, followed by 200 µL of 2% ammonium hydroxide; the plasma mixture was loaded, then washed with 200 µL 5% methanol in 2% ammonium hydroxide and the sample eluted with 50 µL of methanol with 0.1% formic acid into a 96- well plate. Ultrapure water (Barnstead Nanopure, Thermo Fisher-Scientific, Waltham, MA, USA), 50 µL was then added to the 96-well plate and a cap mat placed. The liquid chromatography mobile phase consisted of A: 0.1% formic acid in water and B: acetonitrile with 0.1% formic acid with a flow rate of 0.6 mL/min. The mobile phase started at 95% A at time 0 with a linear gradient to 20% A at 2.8 minutes, back to 95% A at 2.81 minutes with a total run time of 3.5 minutes. A reversed phase C18 column achieved chromatographic separation (Acquity UPLC HSS T3, 2.1x50 mm, 1.8 µM, Waters Corporation). The retention times of butorphanol and the internal standard medetomidine were both 1.6 minutes. The qualifying and quantifying ions for butorphanol and medetomidine were *m/z* (mass to charge ratio) 328.10→310.11 and 201.03→94.94, respectively. Mass spectrometer settings were optimized using the automated tuning feature included in the software which was also used for quantitation (MassLynx 4.1, Waters Corporation). Standard curves in canine plasma were linear between 0.5 and 500 ng/mL and accepted if the coefficient of determination was at least 0.99 and predicted values were within 15% of the actual

concentration, except at 0.5 ng/mL where within 20% of the actual concentration was considered acceptable. The lower limit of quantification was 0.5 ng/mL which had a signal to noise ratio >10. A daily run was accepted if at least 6/9 quality controls were within 15% of the actual concentration and no more than 1 failing QC could occur at the low, middle or high concentration. The interday accuracy and precision were determined on replicates of 5 at each of the following concentrations: low = 1 ng/mL, middle = 50 ng/mL and high = 500 ng/mL. The accuracy at 1, 50 and 500 ng/mL was 100%, 107% and 95% of the actual concentration, respectively. The precision (% coefficient of variation) at 1, 50 and 500 ng/mL was 5, 5 and 6%, respectively.

Pharmacokinetic Analysis

Pharmacokinetic analyses were conducted using computer software and noncompartmental methods (Phoenix, 64, Certara, Princeton, NJ, USA). The area under the concentration-time curve from time 0 extrapolated to infinity (AUC_{inf}), percent of the AUCinf extrapolated from the last measured time point to infinity (AUC extrapolated), maximum plasma concentration (C_{MAX}), time to maximum plasma concentration (T_{MAX}), concentration back extrapolated to time 0 (C0), terminal slope (λz), terminal half-life (T ½ λz), mean residence time extrapolated to infinity (MRT), volume of distribution at steady state (Vss), volume of distribution by area method (Vz) and plasma clearance (Cl) were calculated using the computer software. Terminal slopes were estimated using at least 3 time points on the log-linear terminal slope of the plasma concentration vs. time curve with a $1/y^2$ weighting. The areas under the concentration time curves were estimated using the linear trapezoidal method. For CRI analysis, the steady state plasma concentration (Css) was determined for each dog by averaging the

plasma concentrations for each time point at steady state. The plasma clearance during the IV CRI was calculated by dividing the infusion rate by the Css. The relative percent of the butorphanol dose absorbed (%F) following SC-bicarbonate treatment compared to SC was determined by dividing the dose normalized AUCinf for each formulation multiplied by 100.

Statistical Analysis

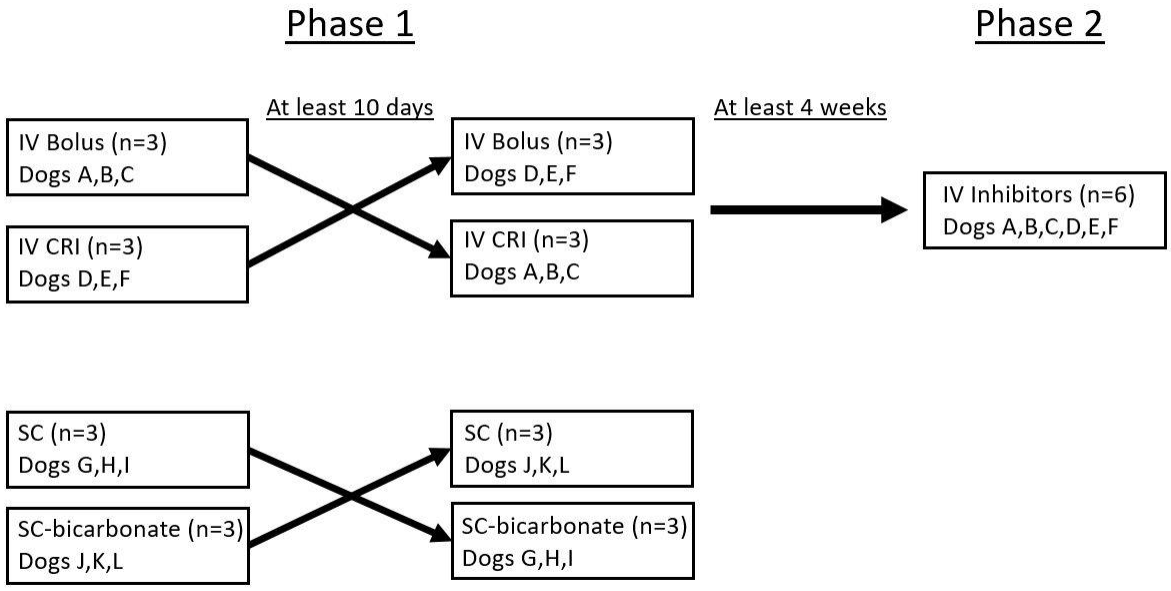
For IV bolus and SC rectal temperature data, an analysis of variance (ANOVA) test was used since the data were normally distributed with equal variance. The rectal temperature data for the IV CRI and sodium bicarbonate group were not normally distributed with uniform variance so a Kruskal-Wallis ANOVA on ranks was used. A significance level of $P<0.05$ was used for all evaluations. Analysis was performed using statistical software (Sigma-Plot, version 12.5, Systat Software Inc, San Jose, California). For the pharmacokinetic parameters of IV bolus, IV with inhibitors and IV CRI plasma samples, the post hoc power analyses were <0.8 (with a $P<0.05$) for the AUCinf, C0 and MRT, therefore statistical analyses were not reported for these pharmacokinetic parameters. For the SC and SC-bicarbonate groups, the post hoc power analyses were <0.8 for all of the pharmacokinetic parameters and were also not reported.

Adverse Effects

Animals were monitored for adverse effects throughout the study period. Any adverse effects were recorded. Adverse effects are reported as the prevalence of observed individual adverse effects recorded within a treatment group and more than one adverse effect may have occurred within the same animal.

Figure 1

Study Design



Flow chart of the study design assessing the pharmacokinetics and pharmacodynamics of butorphanol in dogs (n=6/ per treatment). IV bolus = 0.4 mg/kg IV; IV CRI = 0.2 mg/kg loading dose followed by 0.2 mg/kg/hr for 8 hours; SC = 0.4 mg/kg SC; SC-bicarbonate = 0.8 mg/kg butorphanol SC mixed with an equal volume of 8.4% sodium bicarbonate; IV inhibitors = 0.4 mg/kg IV 1.5-2 hours after the combination of chloramphenicol 50 mg/kg PO, cimetidine 50 mg/kg PO, citalopram 2 mg/kg PO and ketoconazole 20 mg/kg PO

Table 1

Sample Collection and Processing

	Samples collected		
Time	**SC and SC-Bicarbonate**	**IV bolus / IV inhibitors**	**IV CRI**
0 minutes	Blood, RT, sedation	Blood, RT, sedation	Blood, RT, sedation
5 minutes	N/A	Blood	Blood
10 minutes	Blood	Blood	Blood
20 minutes	Blood	N/A	N/A
30 minutes	Blood, RT, sedation	Blood, RT, sedation	Blood, RT, sedation
45 minutes	Blood	N/A	N/A
1 hour	Blood, RT, sedation	Blood, RT, sedation	Blood, RT, sedation
1.5 hours	RT, sedation	RT, sedation	RT, sedation
2 hours	Blood, RT, sedation	Blood, RT, sedation	Blood, RT, sedation
3 hours	RT, sedation	RT, sedation	RT, sedation
4 hours	Blood, RT, sedation	Blood, RT, sedation	Blood, RT, sedation
5 hours	RT, sedation	RT, sedation	RT, sedation
6 hours	Blood, RT, sedation	Blood, RT, sedation	Blood, RT, sedation
7 hours	RT, sedation	RT, sedation	RT, sedation
8 hours	Blood, RT, sedation	Blood, RT, sedation	Blood, RT, sedation
8.5 hours	N/A	N/A	Blood
9 hours	RT, sedation	RT, sedation	Blood, RT, sedation
10 hours	RT, sedation	RT, sedation	Blood, RT, sedation
11 hours	RT, sedation	RT, sedation	RT, sedation
12 hours	Blood, RT, sedation	Blood, RT, sedation	Blood, RT, sedation
24 hours	RT, sedation	RT, sedation	RT, sedation

Sample collection schedule used to assess the pharmacokinetics and pharmacodynamics of butorphanol administered to dogs. IV bolus = 0.4 mg/kg IV; IV CRI = 0.2 mg/kg loading dose followed by 0.2 mg/kg/hr for 8 hours; SC = 0.4 mg/kg SC; SC-bicarbonate = 0.8 mg/kg butorphanol SC mixed with an equal volume of 8.4% sodium bicarbonate; IV inhibitors = 0.4

mg/kg IV 1.5-2 hours after the combination of chloramphenicol 50 mg/kg PO, cimetidine 50 mg/kg PO, citalopram 2 mg/kg PO and ketoconazole 20 mg/kg PO. RT = rectal temperature.

Results

Rectal Temperature

One dog in the CRI group removed its catheter and it was uncertain when the removal occurred; therefore, it was excluded from all data analyses. The mean rectal temperature changes from baseline at predetermined time points are shown in Figure 2. Rectal temperature changes from baseline (mean± SD) for individual treatments; butorphanol IV bolus, IV CRI, SC, and SC-bicarbonate are shown in Figures 4, 5, 6, and 7, respectively. There was a statistically significant decrease in rectal temperature, compared to baseline measurements, for IV bolus (1.5-4 hr), IV CRI (1-5 hr), and SC-bicarbonate (2-7 hr) administration of butorphanol. A statistically significant decrease in rectal temperature did not occur after SC administration. Unfortunately, during the IV butorphanol with CYP inhibitors study, a malfunction with the housing facility's central air conditioning occurred. While supplemental portable air conditioning units were brought in to regain temperature control of the facilities, fluctuating ambient temperatures did occur. Therefore, rectal temperatures are not reported for the IV with CYP inhibitors treatment group.

Sedation

Following IV bolus administration, at least one dog had a sedation score >1 (moderate sedation or higher) from 1.5-2 hours (Figure 3, Table 2). During IV CRI administration, a sedation score >1 in at least one dog was observed from 0.5-8 hours. After SC administration, a sedation score >1 was observed in at least one dog from 1.5-2 hours. For the SC-bicarbonate administration, a sedation score >1 was observed in at least one dog from 1-2 hours. No dog had a sedation score of unresponsive (score of 4) at any time during the study. No dog in any

treatment group had a sedation score >1 after 8 hours. Sedation scores were not recorded in 3 dogs (2 dogs at 24 hours in the IV bolus group and 1 dog at 12 hours in SC-bicarbonate group).

Pharmacokinetics

Plasma concentrations and pharmacokinetic parameters for butorphanol for IV bolus, IV CRI, and IV with CYP inhibitors are summarized in Table 3 and Figure 8. Plasma concentrations and pharmacokinetic parameters for SC and SC-bicarbonate administration are reported in Table 4 and Figure 9. The actual doses of the CYP inhibitors administered (mean with range) were: chloramphenicol 52.5 (41.7-61.7) mg/kg PO, cimetidine 49.3 (45.0-55.6) mg/kg PO, citalopram 2.4 (2.0-3.3) mg/kg PO, and ketoconazole 21.0 (16.7-24.7) mg/kg PO. The geometric mean (range) of the butorphanol plasma concentrations extrapolated to time 0 (C0) after IV bolus and IV with purported CYP inhibitor administration were 182.9 ng/mL (142.7-279.9 ng/mL), and 130.3 ng/mL (110.0-157.8 ng/mL), respectively. The terminal half-lives (T ½ λ*z*) were not significantly different between the IV bolus (mean = 1.47, range 1.19-1.71 hr) and IV CRI groups (1.42 hr, range 0.99-1.83 hr), but both were significantly different compared to the IV inhibitors group (mean = 3.5 hr, range 2.1-10.0 hr, P=0.001). The plasma clearance (Cl) for the IV CRI group (mean=77.3 mL/min/kg, range 63.3-86.7 mL/min/kg) was significantly higher (P<0.001) compared to the IV bolus (mean=46.5 mL/min/kg, range 40.9-61.0 mL/min/kg) and IV with inhibitors (mean= 52.9 mL/min/kg, range 39.5-65.3 mL/min/kg). The clearance was not significantly different (P>0.05) between the IV bolus and IV inhibitor groups. The volumes of distribution (Vz, Vss) were significantly different between the IV bolus and IV with inhibitors. The IV CRI group steady state plasma concentration (Css) had a geometric mean of 43.1 ng/mL and range of (38.5-52.7 ng/mL).

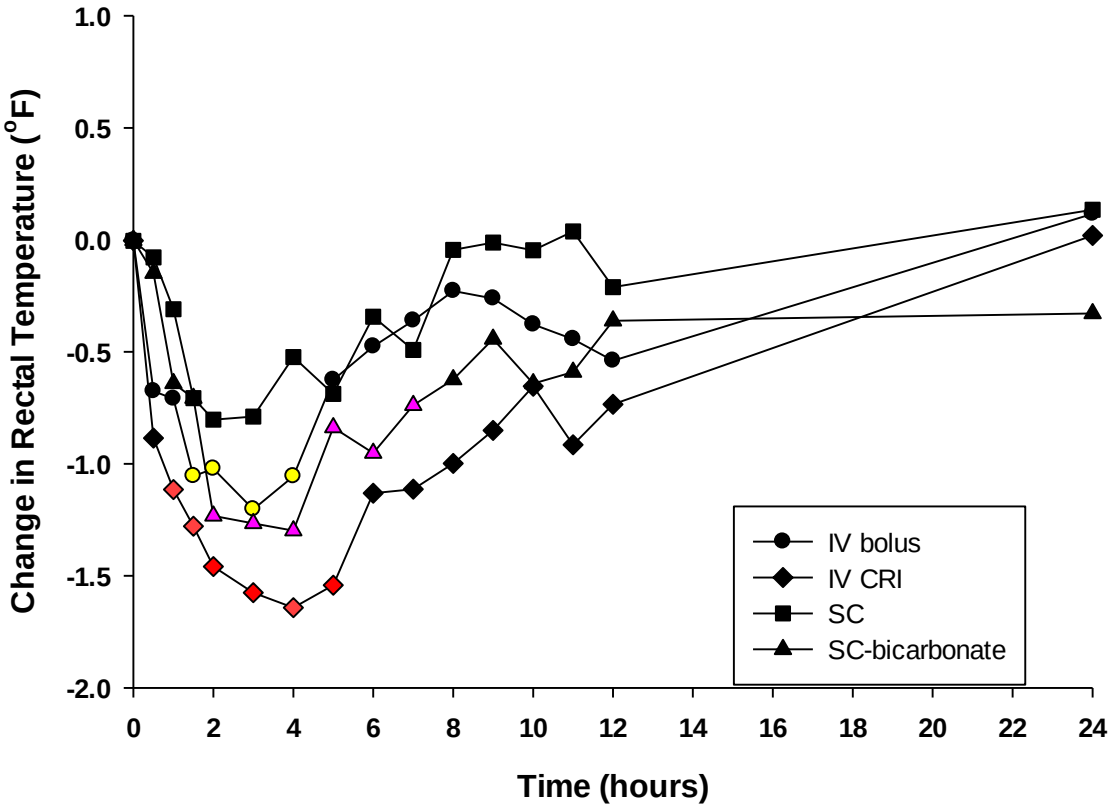
The geometric mean (range) maximum plasma concentration (C_{MAX}) following SC and SC-bicarbonate administration was 74.2 ng/mL (48.6-90.0 ng/mL) and 52.0 ng/mL (8.7-156.2 ng/mL), respectively. The relative bioavailability following SC-bicarbonate administration compared to SC administration was 61% and ranged from 22% to 108%. The geometric mean of the terminal half-life (T ½ λz) of SC and SC-bicarbonate was 1.83 (1.00-5.47) hr and 4.25 (1.65-22.51) hr, respectively. The time to reach maximum plasma concentration (T_{MAX}) after SC and SC-bicarbonate administration was 0.21 (0.17-0.33) hr and 0.22 (0.17-0.50) hr, respectively.

Adverse Effects

Following IV bolus administration, vomiting occurred in 1/6 dogs, and hypersalivation was seen in 1/6 dogs. The IV inhibitors group had 4/6 dogs with vomiting and 4/6 dogs with hypersalivation. During IV CRI administration, vomiting occurred in 1/6 dogs, diarrhea occurred in 1/6 dogs, and hypersalivation occurred in 1/6 dogs. Following SC administration vomiting occurred in 3/6 dogs, an observation of a painful injection occurred in 1/6 dogs, diarrhea occurred in 3/6 dogs, and hypersalivation occurred in 2/6 dogs. After SC-bicarbonate administration, vomiting occurred in 1/6 dogs, diarrhea occurred in 2/6 dogs, and hypersalivation occurred in 1/6 dogs. Diarrhea in all but one dog was characterized by small fecal amounts with mucous. Diarrhea in 1 dog was a large amount of soft stool.

Figure 2

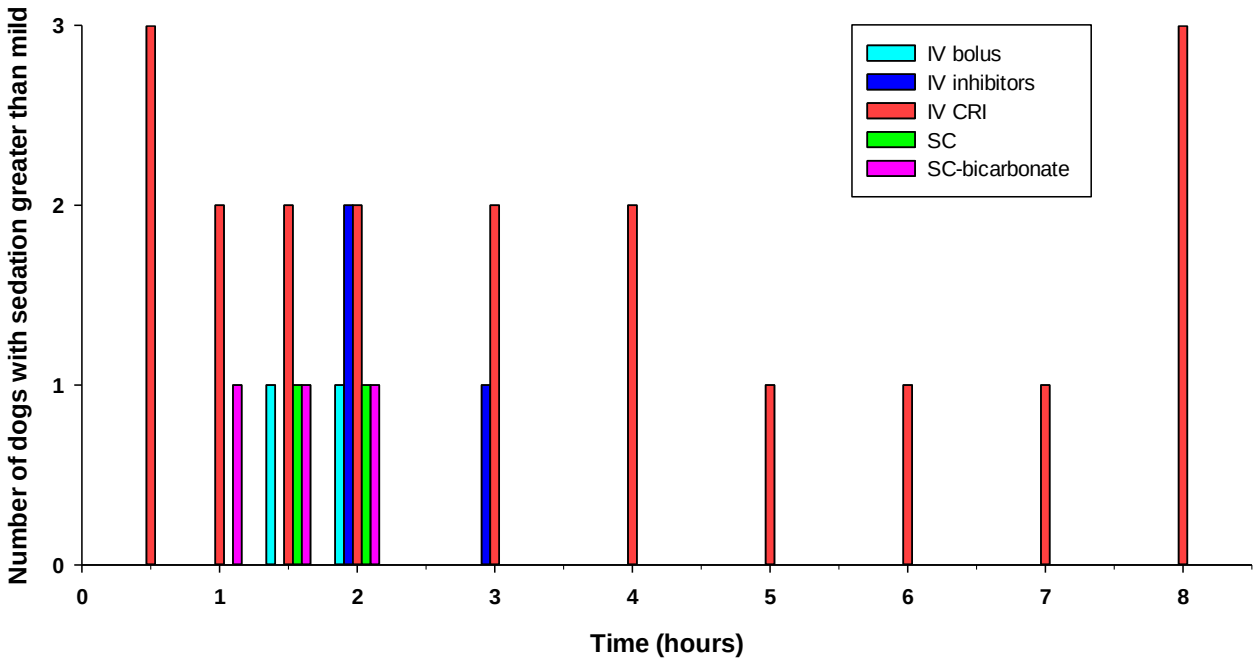
Mean Change in Rectal Tempertature (IV/IV CRI/SC/SC-bicarbonate)



Mean change in rectal temperature (mean) in Fahrenheit (°F) after the administration of butorphanol. Colored symbol indicates significant (P>0.05) difference compared to time 0 within each treatment. See Figure 1 for the treatment key.

Figure 3

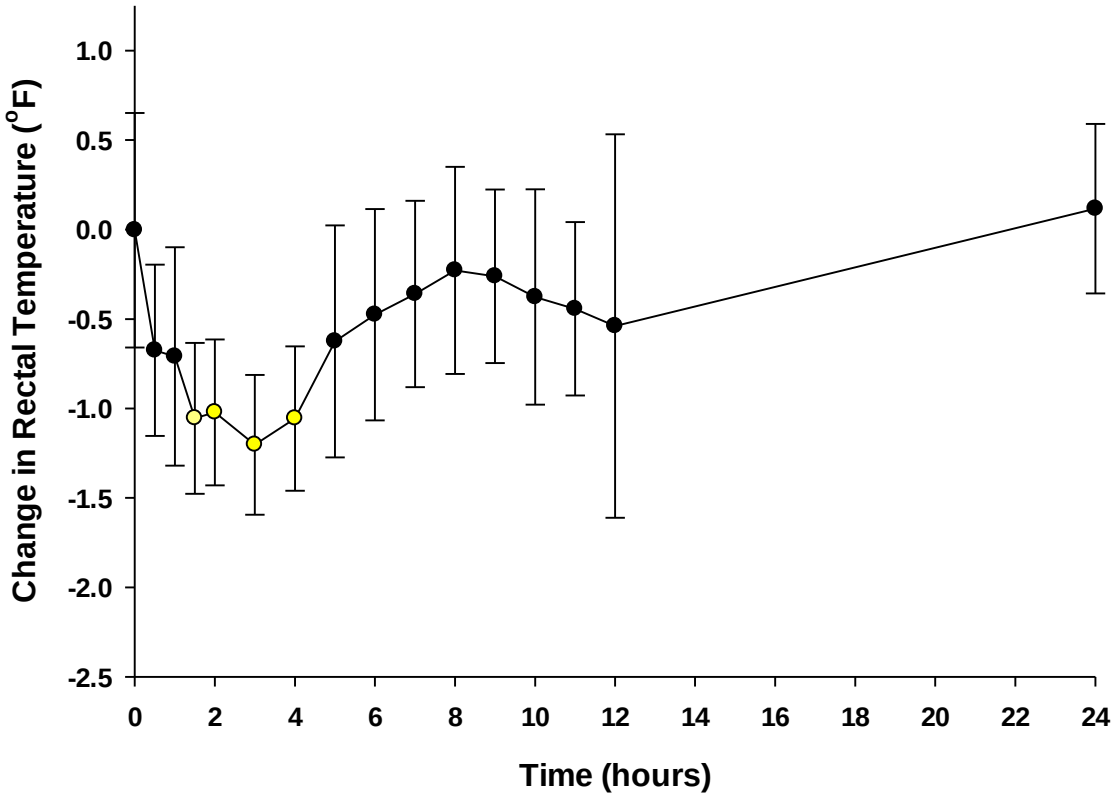
Number of Dogs with Sedation Greater than Mild



The number of dogs at each time with moderate sedation or greater (i.e. number of dogs with sedation score >1). See Figure 1 for treatment key.

Figure 4

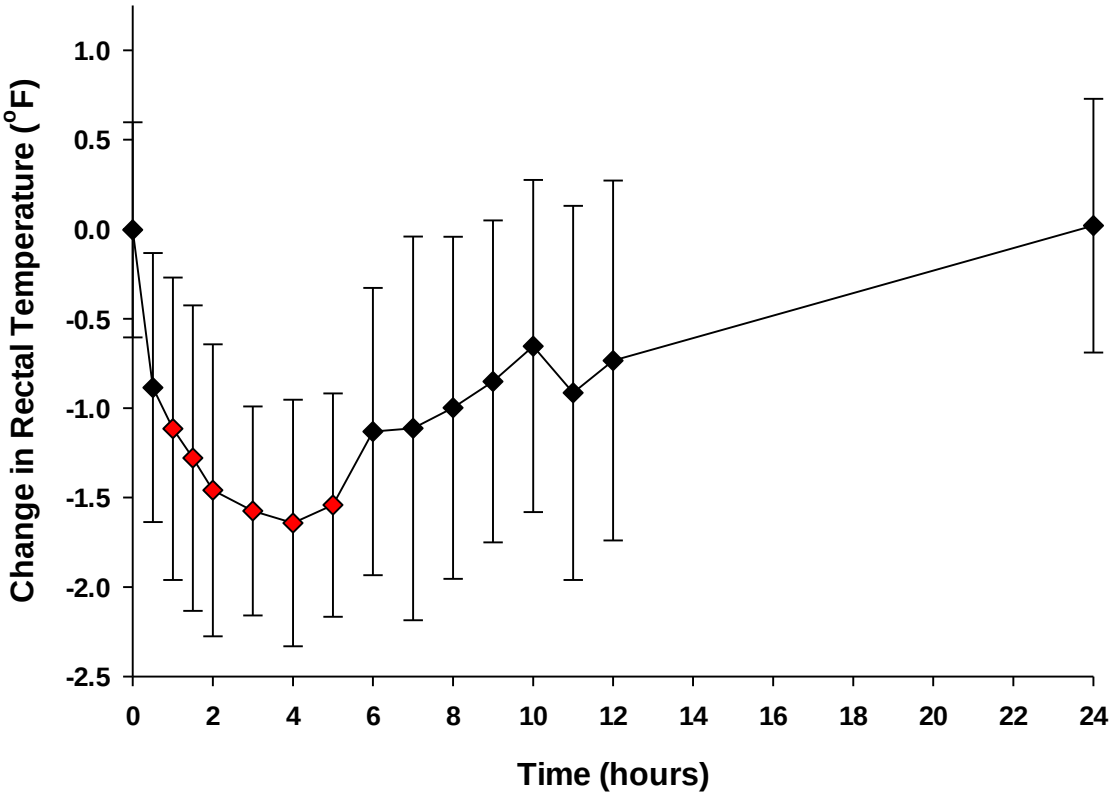
Change in Rectal Temperature (IV)



Change in rectal temperature (mean and standard deviation) in Fahrenheit (°F) after administration of butorphanol IV bolus (0.4 mg/kg IV) to healthy dogs (n=6). The colored symbols indicate statistically significant decreases in rectal temperature when compared to baseline between the 1.5-4 hour time points.

Figure 5

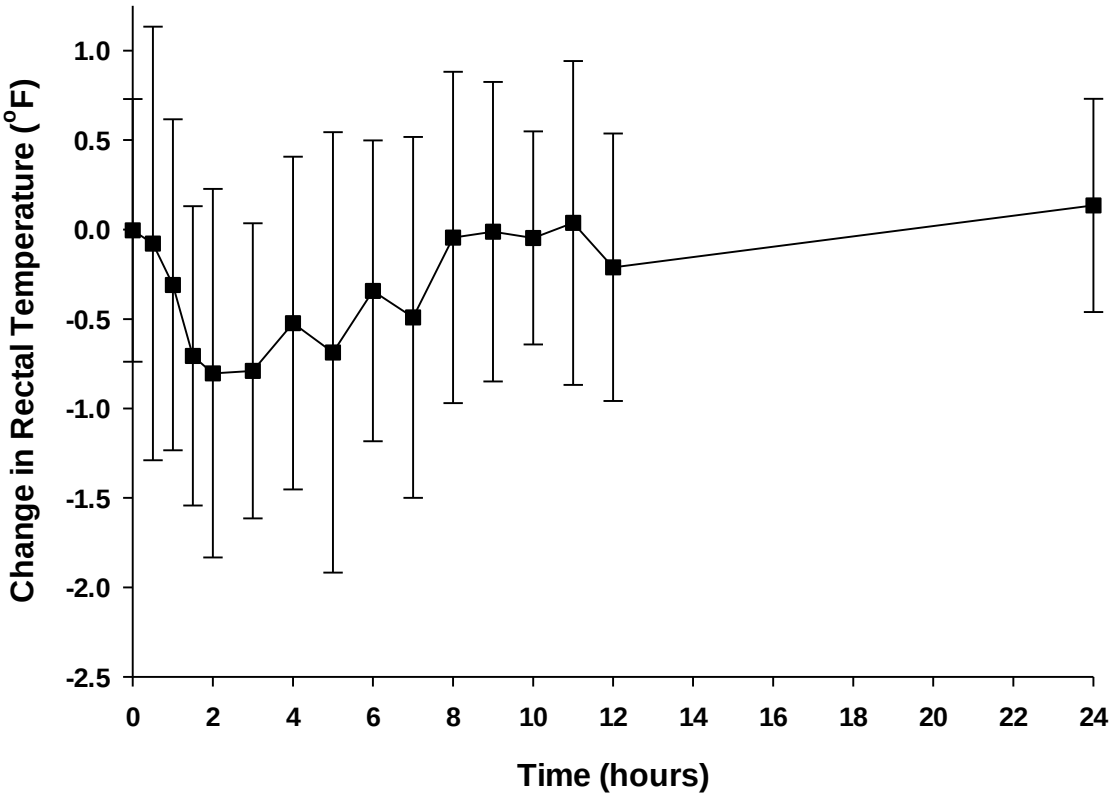
Change in Rectal Temperature (IV CRI)



Change in rectal temperature (mean and standard deviation) in Fahrenheit (°F) after the administration of butorphanol loading dose (0.2 mg/kg IV) and IV CRI (0.2 mg/kg/hr) to healthy dogs (n=5). The colored symbols indicate statistically significant decreases in rectal temperature when compared to baseline between the 1-5 hour time points.

Figure 6

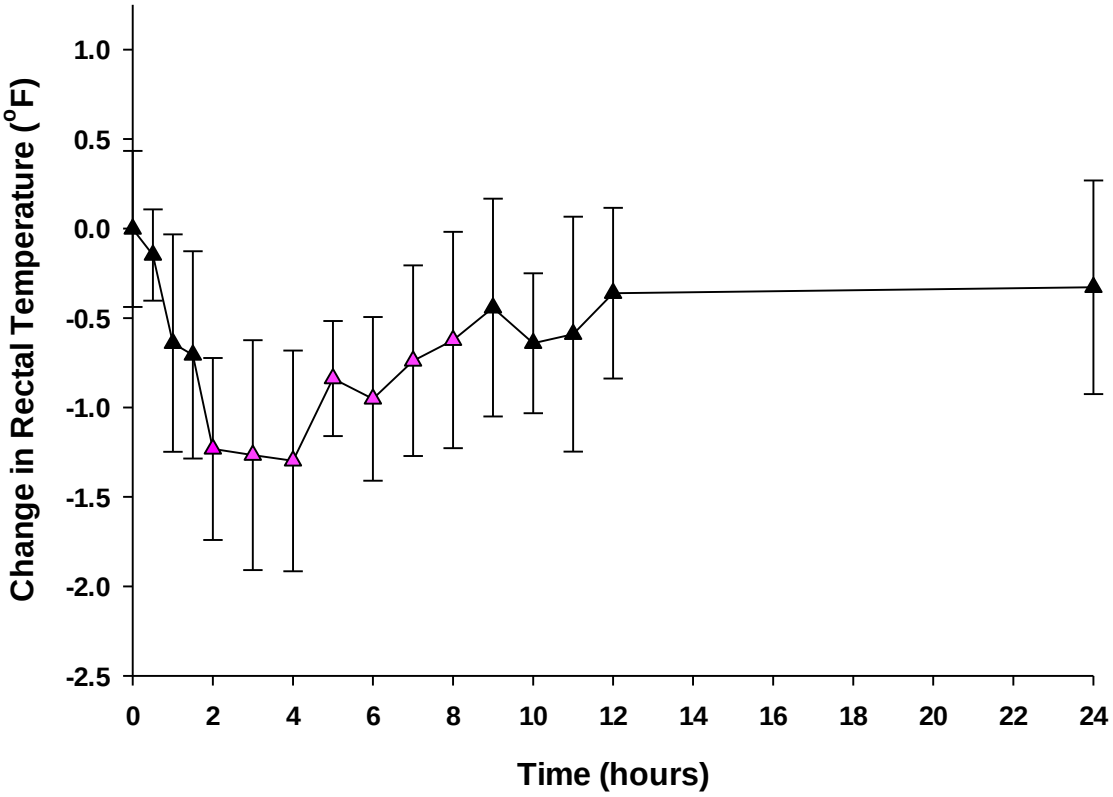
Change in Rectal Temperature (SC)



Change in rectal temperature (mean and standard deviation) in Fahrenheit (°F) after the administration of butorphanol SC (0.4 mg/kg) to healthy dogs (n=6). Statistically significant decreases in rectal temperature when compared to baseline did not occur with this group.

Figure 7

Change in Rectal Temperature (SC-bicarbonate)



Change in rectal temperature (mean and standard deviation) in Fahrenheit (°F) after the administration of SC-bicarbonate butorphanol (0.8 mg/kg butorphanol). The colored symbols indicate statistically significant decreases in rectal temperature when compared to baseline between the 2-7 hour time points.

Table 2

Sedation Score for each Dog per Treatment Group

	IV bolus				IV inhibitors				IV CRI				SC				SC-bicarbonate			
	Sedation Score				Sedation Score				Sedation Score				Sedation Score				Sedation Score			
Time (hr)	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
0	6	0	0	0	6	0	0	0	5	0	0	0	6	0	0	0	6	0	0	0
0.5	0	6	0	0	0	6	0	0	0	3	2	0	0	6	0	0	0	6	0	0
1	0	6	0	0	0	6	0	0	0	3	1	1	0	6	0	0	0	5	1	0
1.5	0	5	1	0	0	6	0	0	0	3	1	1	0	5	1	0	0	5	1	0
2	0	5	1	0	0	4	2	0	0	3	2	0	0	5	1	0	0	5	1	0
3	0	6	0	0	0	5	1	0	0	3	2	0	1	5	0	0	0	6	0	0
4	1	5	0	0	3	3	0	0	0	4	1	0	2	4	0	0	0	6	0	0
5	1	5	0	0	6	0	0	0	0	4	1	0	2	4	0	0	2	4	0	0
6	5	1	0	0	6	0	0	0	0	4	1	0	4	2	0	0	5	1	0	0
7	5	1	0	0	6	0	0	0	0	4	1	0	6	0	0	0	5	1	0	0
8	6	0	0	0	6	0	0	0	0	3	2	0	6	0	0	0	6	0	0	0
9	6	0	0	0	6	0	0	0	0	5	0	0	6	0	0	0	6	0	0	0
10	6	0	0	0	6	0	0	0	0	5	0	0	6	0	0	0	6	0	0	0
11	6	0	0	0	6	0	0	0	2	3	0	0	6	0	0	0	6	0	0	0

12	6	0	0	0	6	0	0	0	4	1	0	0	6	0	0	0	5	0	0	0
24	4	0	0	0	6	0	0	0	5	0	0	0	6	0	0	0	6	0	0	0

The number of dogs within each sedation score category. 0=No Sedation Present; 1=slight sedation; 2=moderate sedation; 3=profound sedation; 4=unresponsive. No sedation scores of 4 were recorded. See Appendix 1 for detailed description of the sedation scoring rubric.

Table 3

Pharmacokinetic Parameters for IV, IV with Inhibitors, and IV CRI

		IV Bolus			IV inhibitors			IV CRI		
Parameter	Units	Geometric Mean	Minimum	Maximum	Geometric Mean	Minimum	Maximum	Geometric Mean	Minimum	Maximum
AUC extrapolated	%	1.0	0.5	2.2	2.9	1.6	9.1	N/A	N/A	N/A
AUC_{inf}	hr*ng/mL	143	109	163	126	102	169	N/A	N/A	N/A
C0	ng/mL	182.9	142.7	279.9	130.3	110.0	157.8	N/A	N/A	N/A
Cl	mL/min/kg	46.5 [a]	40.9	61.0	52.9 [b]	39.5	65.3	77.3 [a,b]	63.3	86.7

λz	1/hr	0.472 [a]	0.406	0.584	0.196 [a,b]	0.069	0.327	0.488 [b]	0.378	0.698
T ½ λz	hr	1.47 [a]	1.19	1.71	3.5 [a,b]	2.1	10.0	1.42 [b]	0.99	1.83
MRT	hr	1.26	1.02	1.67	2.1	1.4	3.6	N/A	N/A	N/A
V_{ss}	L/kg	3.51 [a]	2.64	4.21	6.8 [a]	4.9	14.3	N/A	N/A	N/A
V_z	L/kg	5.91 [a]	5.25	6.67	16.2 [a]	10.5	56.4	N/A	N/A	N/A
C_{ss}	ng/mL	N/A	N/A	N/A	N/A	N/A	N/A	43.1	38.5	52.7

Pharmacokinetic parameters for butorphanol following intravenous (IV) bolus administration alone, administration in combination with purported cytochrome P450 (CYP) inhibitors, or administration as a constant rate infusion (CRI) with an IV loading dose. The post hoc power analyses were <0.8 with a P<0.05 for the AUCinf, C0 and MRT, therefore statistical analyses were not reported for these pharmacokinetic parameters. The same superscript letters indicate significant differences within a row. See Table 1 for key.

Table 4

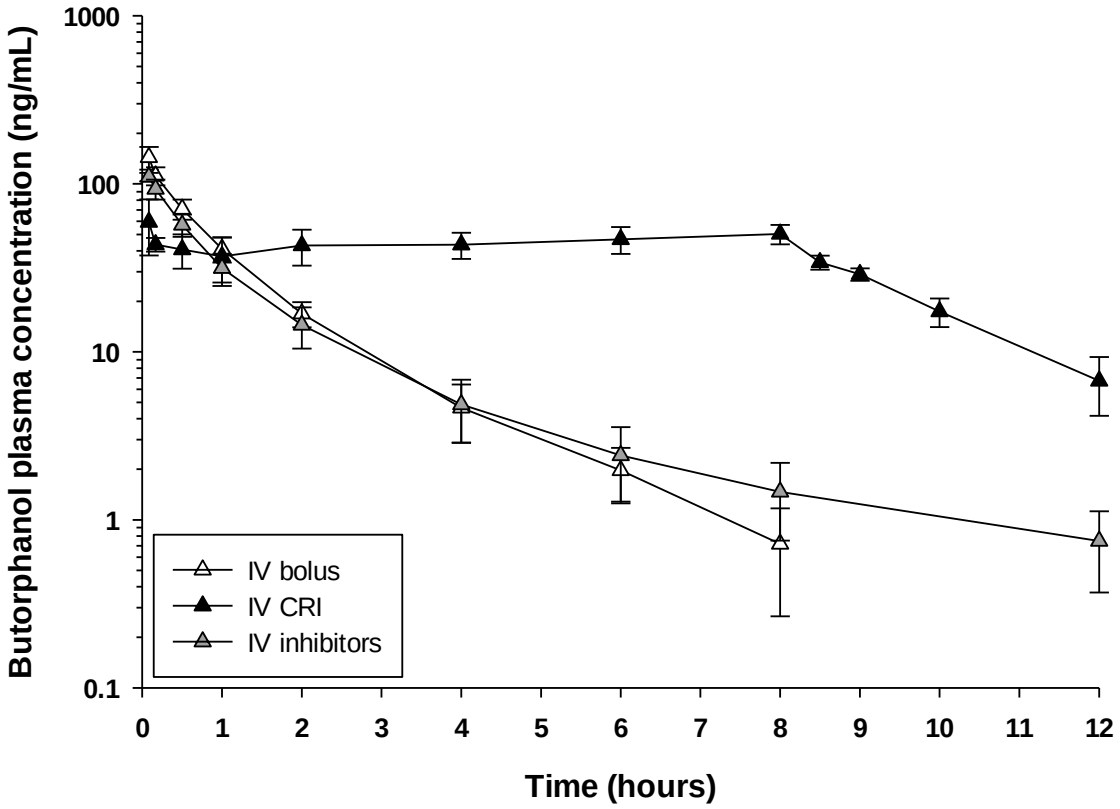
Pharmacokinetic Parameters for SC and SC-bicarbonate

		SC			SC-bicarbonate		
Parameter	Units	Geometric Mean	Minimum	Maximum	Geometric Mean	Minimum	Maximum
$AUC_{extrapolated}$	%	2.5	0.3	22.5	4.6	0.5	63.3
AUC_{inf}	hr*ng/mL	123.3	101.8	152.4	151	58	301
C_{MAX}	ng/mL	74.2	48.6	90.0	52.0	8.7	156.2
T_{MAX}	hr	0.21	0.17	0.33	0.22	0.17	0.50
λz	1/hr	0.378	0.127	0.696	0.163	0.031	0.421
T ½ λz	hr	1.83	1.00	5.47	4.25	1.65	22.51
MRT	hr	2.09	1.24	5.23	4.88	1.94	29.61
F relative	%				61	22	108

Pharmacokinetic parameters for butorphanol following subcutaneous administration, or combined with sodium bicarbonate then administered subcutaneously. The post hoc power analyses were <0.8 with a $P<0.05$ for all of the pharmacokinetic parameters and therefore not reported. See Table 1 for key.

Figure 8

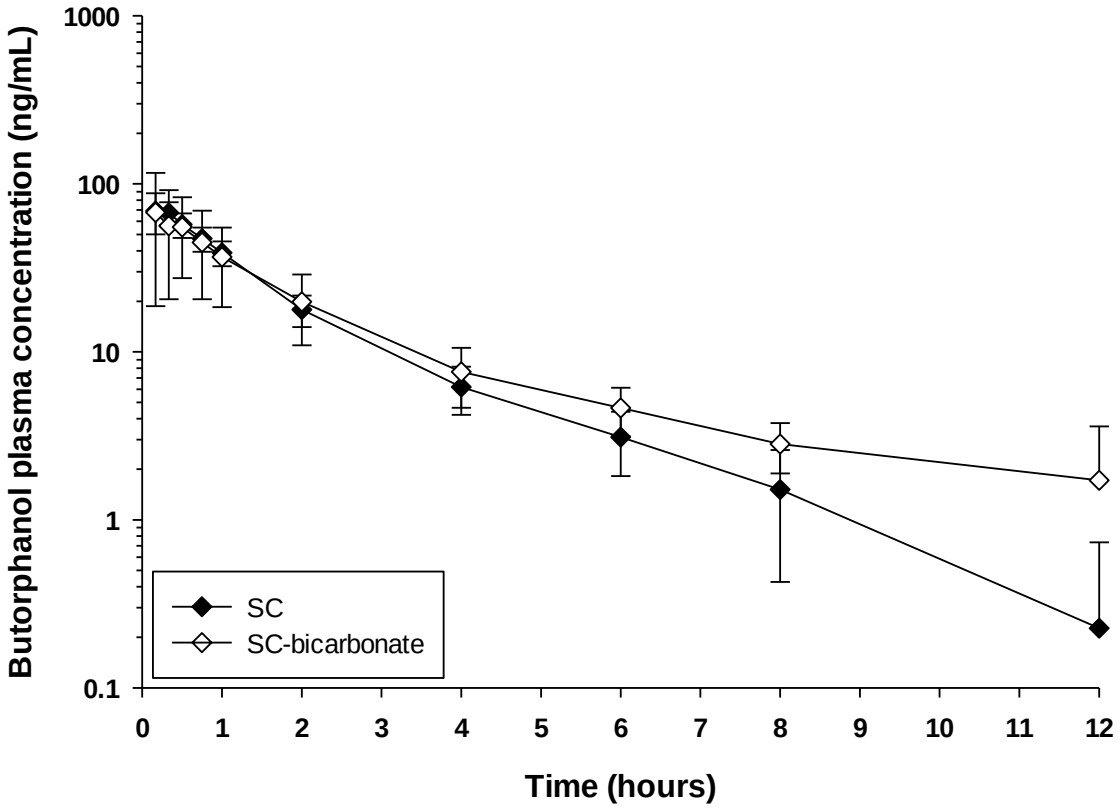
Butorphanol Plasma Concentration (IV/IV with Inhibitors/IV CRI)



Plasma profile (arithmetic mean and standard deviation) of butorphanol administered intravenously. See Figure 1 for key.

Figure 9

Butorphanol Plasma Concentration (SC/ SC-bicarbonate)



Plasma profile (mean and standard deviation) of butorphanol administered subcutaneously. See Figure 1 for key.

Discussion

Butorphanol is an opioid but it has lower potential for abuse and diversion than full μ agonists. As such, establishing a safe and effective analgesic dose will not only provide an animal welfare benefit, but also a One Health benefit by decreasing the use of drugs with high abuse potential (μ opioid agonists). This study was designed to enhance previously reported data (Houghton et al., 1991; Wegner et al., 2008) which demonstrated 0.4 mg/kg IV butorphanol produced significant antinociceptive effects. The study by Wegner et al. also demonstrated butorphanol produced near identical peak antinociceptive effects and duration compared to 0.1 mg/kg IV hydromorphone (Wegner et al., 2008).

A previous study demonstrated dose dependent effects of morphine and methadone on antinociception and decreases in rectal temperature in dogs (Vaupel & Jasinski, 1997). We monitored central opioid effects using rectal temperature as a noninvasive marker in lieu of an induced pain model in order to minimize animal discomfort and stress. Butorphanol induced a significant opioid effect that was measured as a decrease in rectal temperature from baseline in the IV bolus, IV CRI, and SC-bicarbonate groups, but not in the SC group.

The observation of significantly decreased rectal temperature from 1.5-4 hours, in the IV bolus group in the study was similar to the time course of the antinociceptive effect (0.5-4 hours) previously reported using thermal noiciception (Wegner et al., 2008) with both studies using 0.4 mg/kg IV. A colonic distension nociception model documented significant effects 30 minutes (the latest time point assessed) after 0.4 mg/kg IV, but not at doses 0.2 mg/kg IV or lower (Houghton, et, al., 1991). These data, in addition to previously reported data (Vaupel & Jasinski, 1997) suggest that rectal temperature changes induced by butorphanol may be an easy noninvasive method of assessing central opioid effects in preclinical models in dogs. Further

studies simultaneously assessing the effects of butorphanol on rectal temperature and antinociception are needed.

In contrast to the results of the preclinical trials demonstrating significant opioid effects of 0.4 mg/kg butorphanol IV in this study and others (Houghton et al., 1991; Wegner et al., 2008), a clinical trial of 0.4 mg/kg IV butorphanol was reported to provide insufficient analgesia in dogs undergoing soft tissue surgery (Gomes et. al., 2020). However, a major limitation of the study by Gomes was that the intervention point for rescue analgesia in that study (Glasgow Composite Measure Pain Scale total score >3.5) was set below the validated intervention, >5 total score (Gomes et al., 2020, Reid, et. al., 2007). The Glasgow Composite Measure Pain Scale uses six behavioral categories which are not specific for pain and using a low cutoff may not distinguish between postoperative sedation, anxiety and pain. For example, based on the intervention point used by Gomes, a mildly sedated dog who is slow to stand and indifferent to its surroundings, would have been judged as needing rescue analgesia but may not have been painful (Gomes et al., 2020, Reid, et. al., 2007). Therefore, the Gomes study had a bias for treatment failure and false positives for treatment failure which is not indicative of true treatment failure (Gomes et al., 2020). Regardless, clinical trial data using validated methods are needed to confirm the opioid analgesia induced by butorphanol in dogs.

There were some unexpected results in this study. The significant effect of butorphanol on rectal temperature during the IV CRI was lost prior to discontinuing the infusion and despite consistent plasma drug concentrations. There are several explanations why this may have occurred. During the study, the animal caretakers entered into the study room for "welfare checks" between the 6- and 8-hour time points. As seen in Figure 5, the interindividual variability in the rectal temperature increased between 6 and 8 hours. The animals became

visibly excited when they saw the caretakers which may have caused an increase in temperature and is a factor that requires mitigation when using rectal temperature to measure central opioid effects. Interactions with the animal caretakers appear to have affected the IV CRI group to a greater extent than the other groups as the opioid effect was lost in the other groups prior to this disturbance. It is also possible that true pharmacodynamic tolerance developed, but this has not been previously documented to occur within hours, but typically occurs in a time frame from days to weeks (Smith et al., 1999). The rectal temperature data were corrupted during the IV inhibitors treatment due to the air conditioning malfunctioning during the study, also a shortcoming of measuring this parameter.

Another unexpected result was that subcutaneous butorphanol had insignificant effects on rectal temperature. Although this was unexpected, it is similar to a study using a colonic distension nociceptive model in dogs in which doses up to 0.4 mg/kg SC failed to produce a significant response at 45 minutes, but 0.8 mg/kg SC did produce a significant effect (Sawyer et al., 1991). In order for a drug to produce an effect, a drug must reach the target (receptor) in sufficient concentrations for a sufficient period of time (e.g. total exposure). We hypothesize that sufficient concentrations were able to reach opioid receptors in the CNS, but not for a sufficient period of time after SC administration. The C_{MAX} of SC butorphanol was 74.2 ng/mL which exceeded the Css during the IV CRI suggesting sufficient concentrations were achieved. However, butorphanol plasma concentrations rapidly decreased after SC administration which failed to provide exposure for a sufficient amount of time. In comparison, the IV bolus group had much higher drug concentrations after administration (the mean 5 minute plasma concertation was 143.9 ng/mL) which allowed more drug to diffuse into the CNS and increased total exposure to the opioid receptors compared to SC administration. Butorphanol is often administered as part

of many preanesthetic protocols for dogs. However, based on these data collectively (Sawyer et al., 1991), the effective SC butorphanol dose for analgesia is likely greater than 0.4 mg/kg and needs to be better defined.

The SC-bicarbonate group (0.8 mg/kg SC butorphanol) had significant effects on rectal temperature, whereas the SC group (0.4 mg/kg) did not. This is most likely due to the overall higher drug exposure (higher AUCinf) from the higher dose administered. We hypothesized a prolonged effect would occur with a poorly water-soluble suspension, due to drug precipitation, and resulting in a sustained release when administered SC. Based on the pharmacokinetic parameters, the SC-bicarbonate group did not produce a consistent sustained release profile and the greater magnitude and duration of effect was most likely a dose dependent effect. Dose escalation studies would confirm or refute the dose-response hypothesis.

Sedation is a well-known effect of opioids and can be desirable to calm a fractious or anxious animal or could be an adverse effect if normal mentation is desired. Sedation was observed in this study when rectal temperature decreases were not significant. Again, these are similar results to previous studies that reported that the degree of sedation does not correlate with the degree of antinociception and should not be used as an indicator for analgesia (Houghton, et. al., 1991; Sawyer, et. al., 1991). However, if sedation without significant analgesia is needed for procedures that produce little to no pain (e.g. radiographs, ultrasound, etc.), lower doses of butorphanol could have utility.

This is the first study to comprehensively report the pharmacokinetics of butorphanol when administered IV bolus, IV CRI and SC in addition to the novel formulation SC bicarbonate and assessing the effects of purported CYP inhibitors in healthy dogs. The IV pharmacokinetic data are similar to other opioids and the SC data are nearly proportional to previously reported

values (Pfeffer et al., 1980). The addition of bicarbonate to the commercially available butorphanol solution for injection effectively decreased the relative bioavailability and seemed to provide marginal benefits for sustained absorption compared to the commercially available solution. Likewise, the IV inhibitors group had marginal overall effects indicating that, chloramphenicol, cimetidine, citalopram and ketoconazole administered as a single clinically relevant dose 1.5-2 hours prior to butorphanol administration are unlikely to have a clinically meaningful pharmacokinetic interaction. Prior dosing of the inhibitors at 1.5-2 hours prior to butorphanol administration was based on previous studies that documented the T_{MAX} of the oral inhibitors in dogs: chloramphenicol (1.3 hours), cimetidine (1.8 hours), citalopram (1 hour), and ketoconazole (2 hours)(KuKanich & KuKanich 2015; Matsui et al 1995). Further studies with other CYP inhibitors and multiple doses of CYP inhibitors are needed.

The IV loading dose and IV CRI of butorphanol produced consistent effects, but the clearance was greater than expected. This may be due to a truly higher clearance, but could also be an indication of drug loss in the fluid administration set and an overall lower dose administered than expected. Further studies should assess the potential for non-specific drug binding to the infusion set and loss of butorphanol when administered as an IV CRI as described in this study. Regardless, effective concentrations were achieved and maintained over the 8-hour infusion.

Adverse effects, other than sedation, were noted within each treatment group namely vomiting, hypersalivation and diarrhea. Vomiting and hypersalivation are typical adverse effects of opioids in dogs. Diarrhea was an unexpected observation, but based on its appearance most seemed to be stress diarrhea and may be more associated with changes to the dogs' daily routines, transport between long-term housing to the study areas and placement of jugular

catheters versus a true adverse effect to the drug. No interventions were needed for the gastrointestinal adverse events. Painful administration of butorphanol was observed in the SC treatment group (1 dog) and could be due to its acidic formulation or could have been an individual animal response.

As with all studies, this study raises more potential research questions. A future study could assess the dose-response of SC butorphanol at equal doses to SC-bicarbonate which would help discern drug dose versus formulation as the factor for the better response observed in this study. A study directly correlating the response relationships of butorphanol for rectal temperature and antinociception will be helpful in better defining the potential for more widespread use of rectal temperature as a noninvasive surrogate marker for butorphanol antinociception in dogs. The use of doses effective in this study (i.e. 0.4 mg/kg IV and 0.2 mg/kg IV followed by 0.2 mg/kg/hr) should be assessed in clinical patients for analgesia.

Limitations of our study included difficulties with the research facility and its infrastructure which caused an inability to perform the study without outside interferences. For example, interferences from animal caretakers throughout the study and loss of air conditioning during one of the crossovers influenced data collection and results. We also had a larger magnitude of variability within our study for both pharmacokinetic and pharmacodynamics parameters than anticipated indicating a larger sample size is needed (i.e. >6 dogs per treatment) for future studies. Only single doses were assessed for each route/formulation (excluding the IV CRI) and the effects of multiple doses and dose response relationships for each formulation and route would help define the reasons for some of the results we observed.

Conclusion

In conclusion, IV bolus butorphanol (0.4 mg/kg) and a loading dose of butorphanol (0.2 mg/kg IV) followed by a constant rate infusion (0.2 mg/kg/hr) produced central opioid effects. However, 0.4 mg/kg SC butorphanol failed to produce significant opioid effects. The use of butorphanol (0.8 mg/kg) mixed with bicarbonate to form a poorly water soluble butorphanol suspension produced marginal additional effects, but that may have been due to a dose difference. There were minimal effects on the pharmacokinetics of IV butorphanol when administered after a cocktail of purported canine CYP inhibitors.

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Appendix A - Sedation Score Scale (Martinez et al., 2014)

0 - <u>No Sedation Present</u>.

1 - <u>Slight Sedation</u> – almost normal; able to stand easily, but appears somewhat fatigued, subdued or somnolent.

2 - <u>Moderate Sedation</u> – able to stand but prefers to be recumbent; sluggish; ataxic or uncoordinated.

3 - <u>Profound Sedation</u> – unable to rise, but can exhibit some awareness of environment; responds to stimuli through body movement; may be lateral or sternal recumbency.

4 - <u>Unresponsive</u> – in a state of coma or semi-coma from which little or no response can be elicited; remains in lateral recumbency

Appendix B - Master's Defense Presentation

Dosing protocols to increase the efficacy of butorphanol in dogs.

Dariyan Springfield- MS Defense

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Background

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Overview

1. Introduction
2. Methods
3. Results
4. Discussion
5. Conclusion

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Objective, Hypotheses, and Background Information

INTRODUCTION

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Introduction

Need for adequate pain control in dogs

Limitations of NSAID analgesics

There are no FDA approved opioid analgesics for dogs

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Butorphanol

- μ partial agonist/ κ agonist
  - Lower abuse potential
- Widely used opioid analgesic in veterinary medicine
  - For pre-operative and post-operative pain management
  - As an adjunct to anesthesia protocols
- IV butorphanol has near similar peak analgesic effects compared to IV morphine and IV hydromorphone in a preclinical model
  - Butorphanol may have a shorter duration of effect
- DEA Schedule IV drug
- FDA approved in dogs for its antitussive properties

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Pharmacokinetics of Subcutaneous and Intramuscular Butorphanol in Dogs

Pharmacokinetics of Subcutaneous and Intramuscular Butorphanol in Dogs

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Table I—Pharmacokinetic Parameters for Intramuscular and Subcutaneous Butorphanol in Dogs

Parameter	Intramuscular[a]	Subcutaneous[a]
C_{max}, ng/ml	25.1 ± 6.7	33.3 ± 16.9
t_{max}, hr	0.7 ± 0.3	0.5 ± 0.3
AUC, (ng hr)/ml	67.7 ± 16.2	81.7 ± 34.3
$t_{1/2}$, hr	1.53 ± 0.24	1.71 ± 0.40

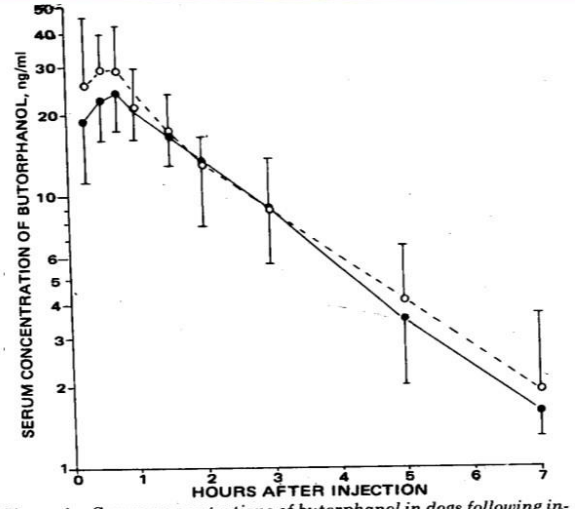

Figure 1—*Serum concentrations of butorphanol in dogs following intramuscular (●) and subcutaneous (○) administration. Mean values from six dogs with standard deviations (vertical bars) are given.*

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Development of a canine nociceptive thermal escape model

Development of a canine nociceptive thermal escape model

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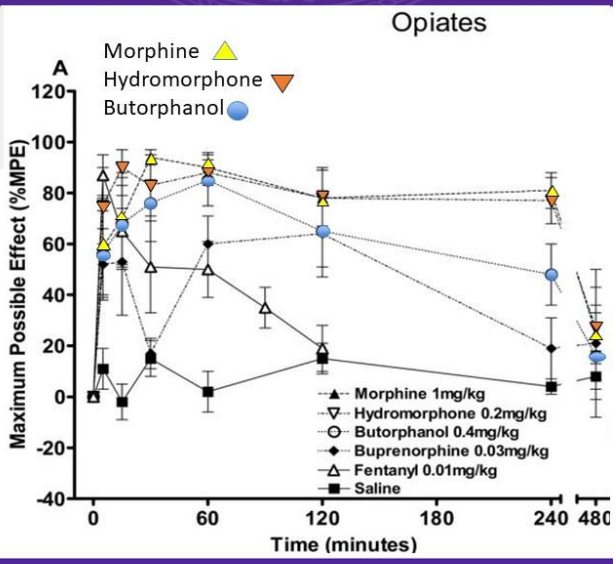
Table 1
Summary of the clinically useful, equi-analgesic doses employed for comparison in the present studies

Drug	Dose (mg/kg)	Reference
Morphine	1.0	KuKanich et al. (2005)
Acepromazine	0.1	Hofmeister and Egger (2005)
Buprenorphine	0.03	Slingsby et al. (2006)
Butorphanol	0.4	Fox et al. (1998)
Dexmedetomidine	0.01	Murrell and Hellebrekers (2005)
Fentanyl	0.01	Sano et al. (2006)
Hydromorphone	0.2	Pettifer and Dyson (2000)



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Opioid Epidemic Statistics

- 75% of drug overdose deaths in 2020 involved an opioid
- More than 564,000 people died from overdoses involving an opioid from 1999-2020
- Overdoses involving an opioid in 2020 nearly killed 68,000 people
  - 82% of these deaths were due to a synthetic opioid

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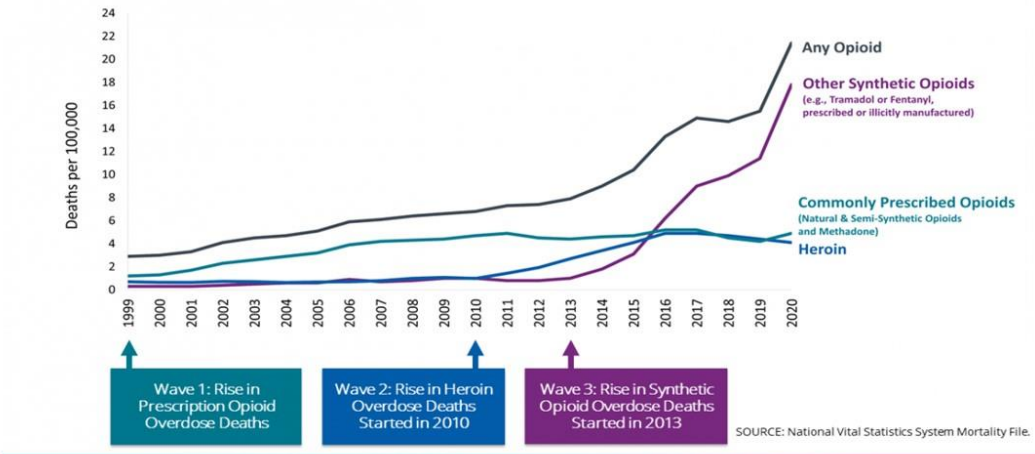
https://www.cdc.gov/opioids/basics/epidemic.html

Three Waves of Opioid Overdose Deaths



SOURCE: National Vital Statistics System Mortality File.

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https://www.cdc.gov/opioids/basics/epidemic.html

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https://www.cdc.gov/opioids/basics/epidemic.html

Objectives

Primary Objective:

- Document the pharmacokinetics and duration of opioid effects of butorphanol after different dosing routes and in combination with other commercially available drugs

Long Term Goal:

- Develop a protocol to produce prolonged effects with the currently available injectable formulation

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Hypotheses

- Intravenous(IV)/subcutaneous (SC) administration of butorphanol will provide significant effects for 1-2 hr
- Consistent and prolonged opioid effects will occur when butorphanol is administered with an IV loading dose and constant rate infusion(CRI)
- Butorphanol administered SC as a suspension will result in slower absorption and prolonged effects
- Cytochrome P450 (CYP) inhibitors will prolong half-life and clinical effects

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Study Design and Analyses

MATERIALS AND METHODS

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Methods

- Measure Opioid Effect:

- Measuring rectal temperature: Decrease in temperature parallels with the antinociceptive effect of opioids (Vaupel & Jasinski 1997)(Gomes et al 2018)

- Measured Sedation: Common side effect or desirable effect seen when using opioids

- Collected plasma samples to determine pharmacokinetic data

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Study Design

12 Healthy Beagles (6 dogs per treatment)

Age = Approx. 1yr
Weight= 5.4-9.8kg

Incomplete Block Design

Phase 1- Group 1:	Phase 1 - Group 2:	Phase 2 :
Butorphanol IV- 0.4mg/kg once Butorphanol CRI- 0.2 mg/kg IV loading dose with constant rate infusion at 0.2mg/kg/hr for 8hrs Total dose= 1.8 mg/kg over 8hr Randomized Complete Block Crossover	Butorphanol SC- 0.4mg/kg once Butorphanol SC-bicarbonate suspension- 0.8 mg/kg mixed with equal volume of 8.4% sodium bicarbonate once Randomized Complete Block Crossover	Chloramphenicol 50 mg/kg PO (CYP 2B11) Cimetidine 50 mg/kg PO (CYP ?) Citalopram 2 mg/kg PO (CYP 2D15) Ketoconazole 20 mg/kg PO (CYP 3A12/26) Given 1.5-2 hr prior to IV Butorphanol (0.4 mg/kg)

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Sample Collection and Processing

- Rectal temperature, sedation and blood were collected throughout the study
- Blood was collected from an aseptic jugular catheter placed 24 hours prior to drug administration
  - A second catheter was placed in the CRI group for drug administration
- Blood was placed in heparin tubes and placed on ice
- Centrifuged and stored frozen at -80 degrees Celsius

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Plasma butorphanol concentration

Plasma butorphanol concentrations were determined by liquid chromatography with triple quadrupole mass spectrometry adapted from a previous study (Paine et al. 2020)

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Key Pharmacokinetic Parameters

- T1/2= elimination half-life (hr)
- Cl= Plasma Clearance (mL/min/kg)
- Vz= Volume of distribution (L/kg)
- AUC= area under the curve (hr*ng/ml)
- F = relative bioavailability (%)
- CMAX= maximum concentration of drug (ng/mL)
- TMAX= time to reach maximum concentration of drug (hr)

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Pharmacokinetic analysis

- Pharmacokinetic analysis was conducted using computer software and non-compartmental methods
- For the CRI group steady state plasma concentration was measured (C_{ss})
- The fraction of the dose absorbed was calculated by dividing the dose normalized AUC_{inf} for SC/SC-bicarbonate and IV/IV with inhibitors

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Rectal Temperature Statistical Analysis

- ANOVA test used for IV and SC rectal temperature since data were normally distributed with equal variance
- Kruskall-Wallis test on ranks was used for the CRI and SC-bicarbonate group since the data were not normally distributed with uniform variance

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Pharmacokinetic Statistical Analysis

- The post hoc power analyses for the PK parameters of IV bolus, IV with inhibitors, and IV CRI groups was <0.8 with a $p<0.05$ for the AUCinf, C0 and MRT, therefore statistical analyses were not reported for these pharmacokinetic parameters
- The post hoc power analyses for the SC and SC-bicarbonate groups was <0.8 with a $p<0.05$ for all of the pharmacokinetic parameters and therefore not reported.

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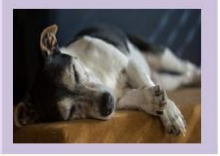
Sedation Scale

0 – No Sedation Present	1 – Slight Sedation	2 – Moderate Sedation	3-Profound Sedation	4-Unresponsive
	Almost normal Able to stand easily Appears somewhat fatigued	Able to stand Sluggish Ataxic or uncoordinated 	Unable to rise, can exhibit some awareness of environment Responds to stimuli through body movement Maybe lateral; sternal recumbency	In a state of coma or semi-coma Little or no response can be elicited 

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Martinez, et al. J. Vet. Pharmacol. Therap. 37(2014)394-405 doi:10.1111/jvp.12096.

Presentation of the following results

RESULTS

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Pharmacokinetic parameters for butorphanol for IV, IV with Inhibitors, and IV CRI

		IV Bolus			IV inhibitors			CRI		
Parameter	Units	Geometric Mean	Min	Max	Geometric Mean	Min	Max	Geometric Mean	Min	Max
Cl	mL/min/kg	46.5 [a]	40.9	61.0	52.9 [b]	39.5	65.3	77.3 [a,b]	63.3	86.7
T ½ λz	hr	1.47 [a]	1.19	1.71	3.5 [a,b]	2.1	10.0	1.42 [b]	0.99	1.83
V_{ss}	L/kg	3.51 [a]	2.64	4.21	6.8 [a]	4.9	14.3	N/A	N/A	N/A
V_z	L/kg	5.91 [a]	5.25	6.67	16.2 [a]	10.5	56.4	N/A	N/A	N/A
C_{ss}	ng/mL	N/A	N/A	N/A	N/A	N/A	N/A	43.1	38.5	52.7

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Pharmacokinetic parameters for butorphanol for SC and SC-bicarbonate

		SC			SC-bicarbonate		
Parameter	Units	Geometric Mean	Minimum	Maximum	Geometric Mean	Minimum	Maximum
CMAX	ng/mL	74.2	48.6	90.0	52.0	8.7	156.2
TMAX	hr	0.21	0.17	0.33	0.22	0.17	0.50
T ½ λz	hr	1.83	1.00	5.47	4.25	1.65	22.51
F relative	%				61	22	108

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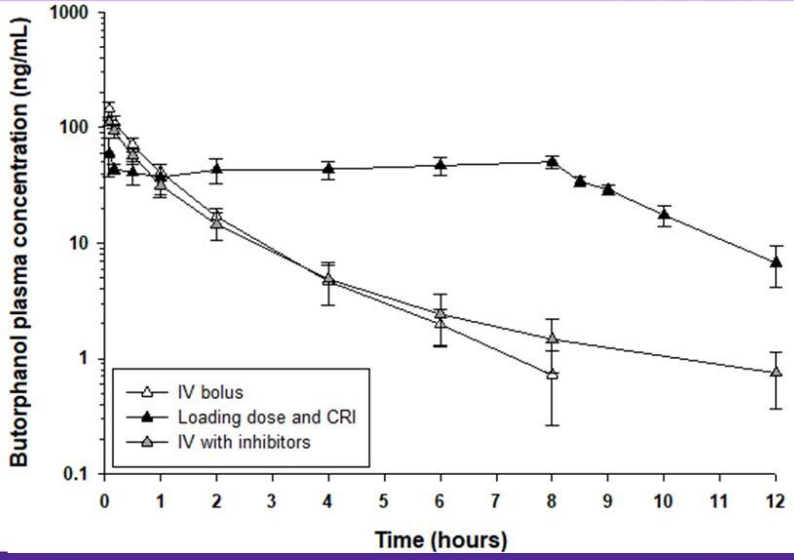
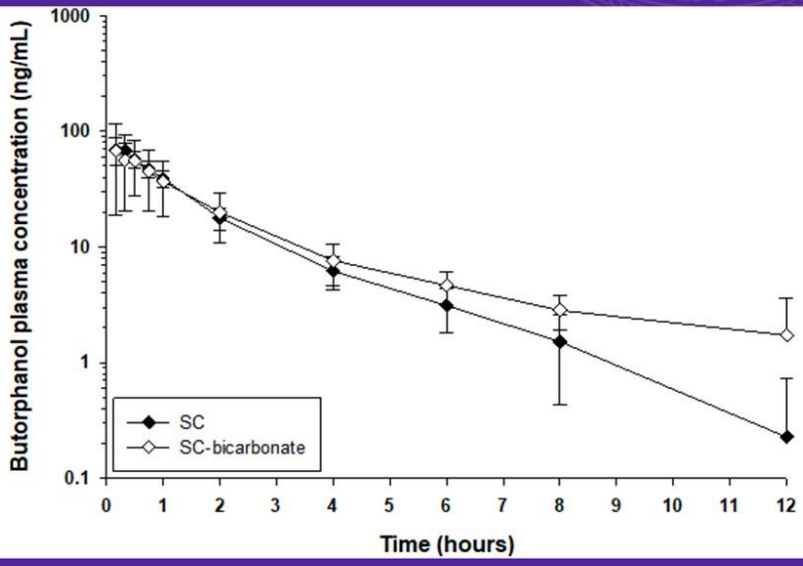
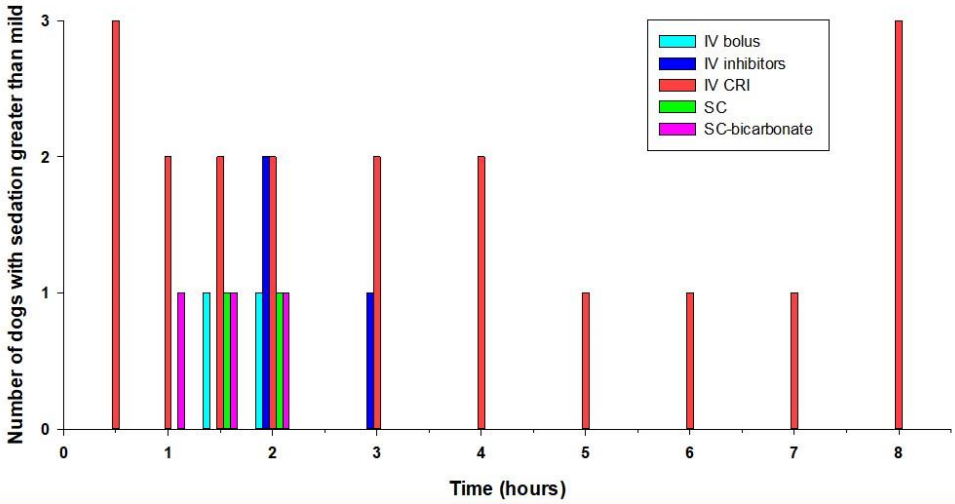


KANSAS STATE UNIVERSITY Plasma Concentration Profile for IV/CRI/IV with Inhibitors



KANSAS STATE UNIVERSITY Plasma Concentration Profile for SC/SC-bicarbonate

Sedation Score(number of dogs greater than mild)



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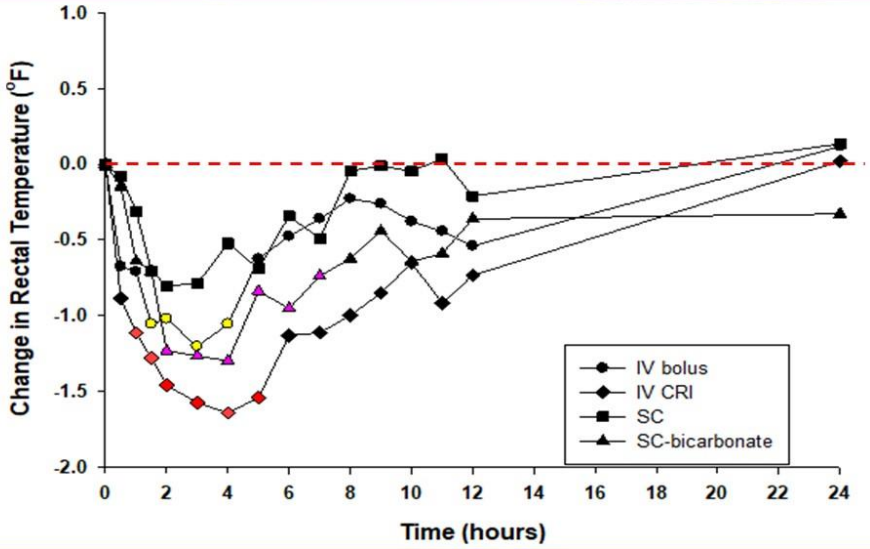
Mean Change in Rectal Temperature- All



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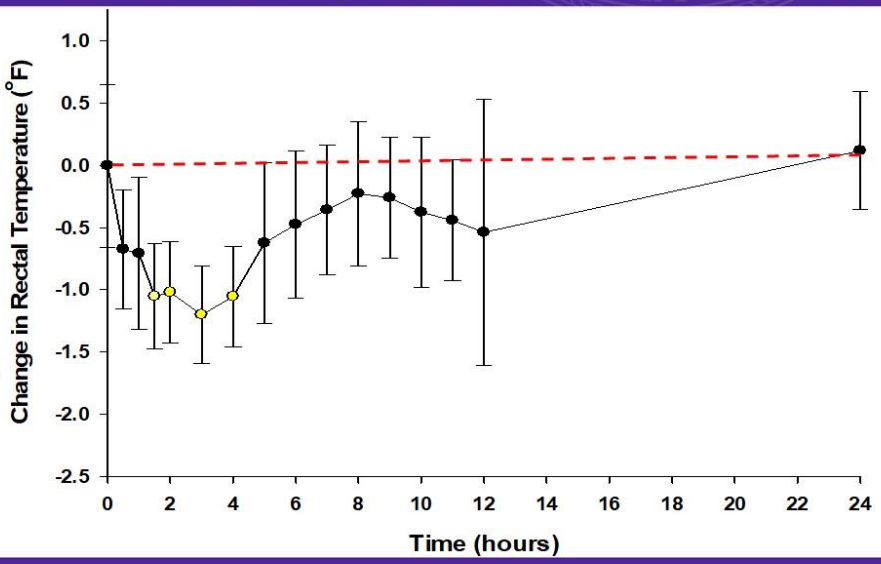
Change in Rectal Temperature (Mean/SD) -IV

p<0.05 (ANOVA)

= Relative to baseline



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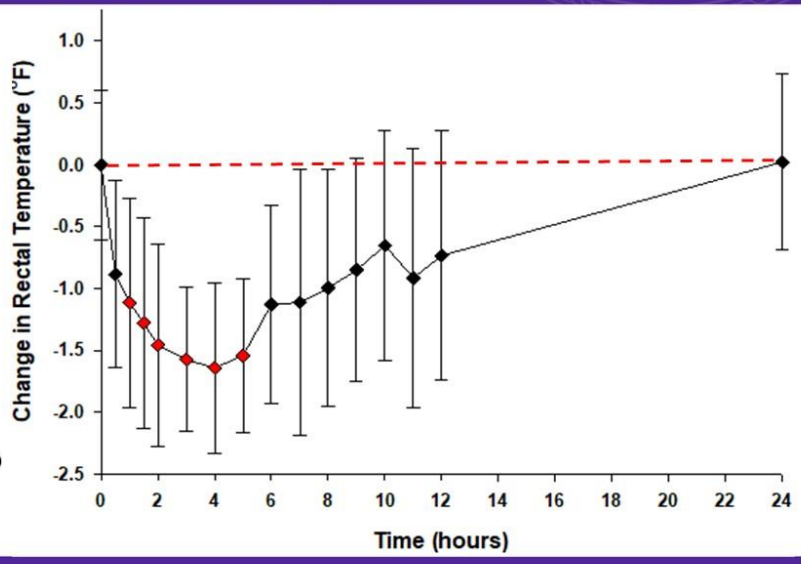
Change in Rectal Temperature (Mean/SD) -CRI

p<0.05 (Kruskall Wallis ANOVA)

= Relative to baseline



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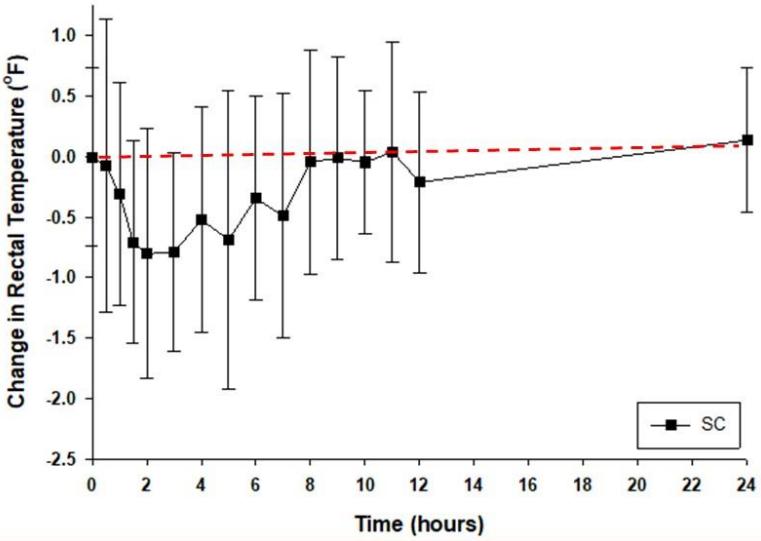
Change in Rectal Temperature (Mean/SD) -SC



ANOVA

- - - - =Relative to baseline

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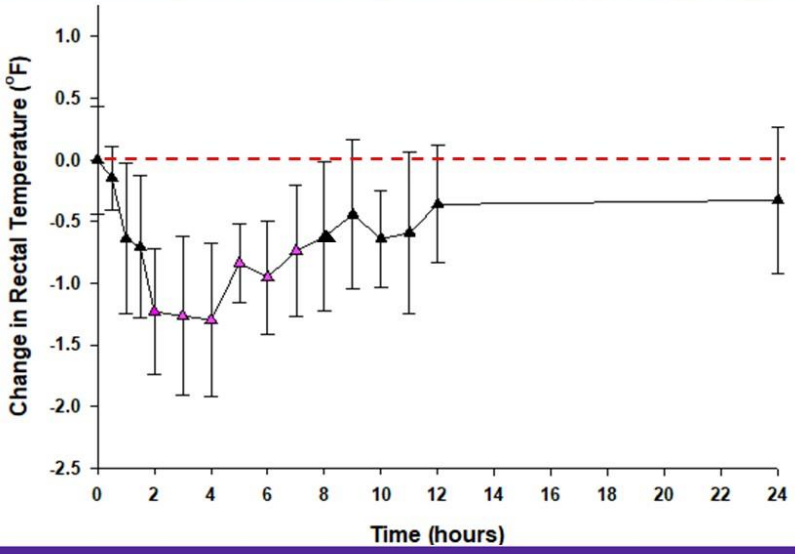
Change in Rectal Temperature (Mean/SD) -SC-bicarbonate



▲ p<0.05 (Kruskall Wallis ANOVA)

- - - - =Relative to baseline

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Adverse Effects

	IV	CRI	IV w/ Inhibitor	SC	SC-bicarbonate
Vomiting	1/6	1/6	4/6	3/6	1/6
Diarrhea	0/6	1/6	0/6	3/6	2/6
Hypersalivation	1/6	1/6	4/6	2/6	1/6
Pain upon Injection	0/6	0/6	0/6	1/6	0/6

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Results Summary

- Sedation was observed between all groups
- Significant decrease in RT for IV,CRI and SC-bicarbonate ($p<0.05$)
  - However, SC did not produce a significant decrease in RT
- Sedation outlasted rectal temperature and is an unreliable marker for analgesia
  - (Houghton et al 1991; Sawyer et al 1991)
- Some adverse effects are commonly seen with opioid use
  - Other adverse effects may have been stressed induced

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Results Summary cont.

- CRI produced consistent and prolong plasma concentrations for the duration of the infusion
- IV group produced central opioid effects as seen in previous studies
- IV Inhibitors group produced some effects but not to the extent as hypothesized
- SC administration had a rapid decrease in plasma concentration leading to failure in sufficient time of exposure
- SC-bicarbonate did not produce a sustained release as hypothesized
  - The greater duration and magnitude of effects are potentially dose related

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Limitations, Future Studies and Conclusion

DISCUSSION

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Discussion

This is the first study to comprehensively report the pharmacokinetics of butorphanol when administered IV, CRI,SC in a novel formulation and IV in combination with CYP Inhibitors

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Limitations

- Difficulties with the research facility and infrastructure
  - This caused an inability to perform the study without interference
- Larger magnitude of variability for our PK/PD parameters than anticipated
  - Indication for the need of a larger sample size (> 6 dogs per treatment) for future studies
- Only single doses were administered
  - Multiple doses/dose response could help define reasons for observed results

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Future Studies

1. Looking at SC butorphanol and SC-bicarbonate at equal doses to discern drug dose vs formulation factors

2. Investigate the effectiveness of 0.4mg/kg IV and 0.2 mg/kg IV loading with 0.2mg/kg/hr CRI in clinical trials

3. Dose escalation study in clinical patients for SC butorphanol to determine dose response

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Conclusion

- IV Butorphanol and CRI of butorphanol with an IV loading dose produced central opioid effects at these doses
  - However SC butorphanol failed to produce significant opioid effects
- Butorphanol mixed with bicarbonate produced marginal additional effects
  - Potentially due to a difference in dose
- Minimal effects of pharmacokinetics of IV butorphanol with CYP inhibitor cocktail

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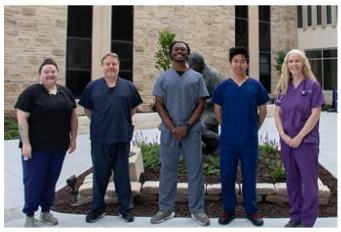

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Glossary of Terms

IV- Intravenous bolus of butorphanol

CRI- Constant Rate of Infusion

IV inhibitors- Intravenous bolus concurrently administered with cytochrome P450 enzyme inhibitors

IM- Intramuscular administration of butorphanol

SC- Subcutaneous

SC-bicarbonate- Subcutaneous administration of butorphanol mixed with sodium bicarbonate within the same syringe

RT- Rectal temperature

CYP- Cytochrome P450 enzyme

AUCinf- The area under the curve extrapolated to infinity

AUC extrapolated- The percent of the AUCinf extrapolated

C_{MAX}- maximum plasma concentration

T_{MAX}- time to maximum plasma concentration

C0- concentration back extrapolated to time 0

λz- terminal slope

T ½ λz- terminal half-life

MRT- mean residence time extrapolated to infinity

Vss- volume of distribution at steady state

Vz- volume of distribution by area method

Cl- plasma clearance

Css- the steady state plasma concentration

%F- The relative percent of butorphanol absorbed into systemic circulation