Pharmacokinetics of buprenorphine after intravenous and oral transmucosal administration in guinea pigs (Cavia porcellus)

Miranda J. Sadar DVM
Heather K. Knych DVM, PhD
Tracy L. Drazenovich DVM
Joanne R. Paul-Murphy DVM

OBJECTIVE
To determine pharmacokinetics and sedative effects of buprenorphine after IV and oral transmucosal (OTM) administration in guinea pigs.

ANIMALS
14 male guinea pigs (6 adults for preliminary experiment; eight 8- to 11-week-old animals for primary study).

PROCEDURES
A preliminary experiment was conducted to determine an appropriate buprenorphine dose. In the primary study, buprenorphine (0.2 mg/kg) was administered IV or OTM, and blood samples were obtained. The pH of the oral cavity was measured before OTM administration. Sedation was scored for 6 hours on a scale of 0 to 3 (0 = no sedation and 3 = heavy sedation). After a 7-day washout period, procedures were repeated in a crossover manner. Plasma buprenorphine concentration was quantified, and data were analyzed with a noncompartmental pharmacokinetic approach.

RESULTS
Mean peak plasma buprenorphine concentrations were 46.7 and 2.4 ng/mL after IV and OTM administration, respectively. Mean time to maximum plasma buprenorphine concentration was 1.5 and 71.2 minutes, and mean terminal half-life was 184.9 and 173.0 minutes for IV and OTM administration, respectively. There was a range of sedation effects (0 to 2) for both routes of administration, which resolved within the 6-hour time frame.

CONCLUSIONS AND CLINICAL RELEVANCE
On the basis of pharmacokinetic parameters for this study, buprenorphine at 0.2 mg/kg may be administered IV every 7 hours or OTM every 4 hours to maintain a target plasma concentration of 1 ng/mL. Further studies are needed to evaluate administration of multiple doses and sedative effects in guinea pigs with signs of pain. (Am J Vet Res 2018;79:260–266)

Treatment and prevention of pain in exotic companion mammals, such as guinea pigs, may be challenging because of a lack of data available on the safety and efficacy of analgesics. The standard of practice is to provide pain relief to animals for conditions considered painful in humans, but many dosage recommendations are based on the perceived response to treatment, clinical experience, and lack of observable toxic effects; however, these extrapolations are difficult to interpret because of numerous factors. The lack of information on dose-response characteristics and dosing intervals for analgesic agents often compels veterinarians to extrapolate information from studies performed in other species.

Opioids are frequently used in veterinary medicine and are considered the most effective class of analgesic drugs for perioperative pain. Opioids are a diverse group of drugs with morphine-like activity that bind reversibly to specific receptors in the CNS and peripheral nervous system, which modifies the transmission and perception of pain in all vertebrate species that have been evaluated. Opioid receptors in the CNS and peripheral nervous system are classified into 3 major categories (ie, µ-, κ-, and δ-opioid receptors) as well as the orphan opioid-like receptor. The action of opioid drugs on these receptors activates G-coupled proteins, which leads to a reduction of transmission of nerve impulses and inhibition of neurotransmitter release. Buprenorphine is a partial µ-opioid receptor agonist, but its action on the κ-opioid receptor is less clear. Buprenorphine has a ceiling effect in mammals (increases in dosages do not result in improved analgesia) or a bell-shaped dose-response curve in which higher dosages can have a lower analgesic effect. When assessing analgesia after administration

ABBREVIATIONS
AUC Area under the plasma concentration-versus-time curve
Cmax Maximum plasma concentration
λz Terminal rate constant
OTM Oral transmucosal
Tmax Time to maximum plasma concentration
of buprenorphine, plasma concentrations ≥ 1 ng/mL have been considered to be analgesic on the basis of results for studies in various species, including humans,11 dogs,12 cats,13,14 and rats.15 The primary routes of buprenorphine administration for dogs and cats are parenteral and OTM, which provide a slow-onset, long-acting, μ-opioid receptor agonist effect for the treatment of pain of mild to moderate severity.16 The OTM route is effective in dogs,17 cats,18 rats,19 and humans.20 The OTM route is frequently used for cats, and it has allowed veterinarians and owners to provide opioids for pain relief to animals in their home environment.13,15,18,21 Salivary pH may alter the absorption of buprenorphine (which is a weak base with a pKa of 8.24) by dictating the degree of ionization, and a more basic pH of the saliva may increase the extent, and potentially decrease the variability, of absorption.22 To the authors’ knowledge, the oral pH of guinea pigs has not been reported.

Few studies23–26 have been conducted to evaluate the nociceptive or analgesic properties of buprenorphine in guinea pigs. In most of those studies, analgesia was measured through perceived improvement or lack of appreciable adverse effects and not on the basis of pharmacokinetic or pharmacodynamic information. Published doses of buprenorphine for guinea pigs range from 0.005 to 0.22 mg/kg, and the routes of administration include SC, IM, and injection into the oral cavity.23–26 Therefore, the purpose of the study reported here was to determine whether IV and OTM administration of a selected dose of buprenorphine would provide appropriate plasma concentrations, compared with those in other species for which analgesic concentrations have been established, and to evaluate the sedative effects of buprenorphine in guinea pigs.

Materials and Methods

Animals

Fourteen sexually intact male Hartley guinea pigs were used (6 were used for a preliminary experiment, and 8 were used in the subsequent primary experiment). Guinea pigs used in the preliminary experiment were adults (exact age unknown) that were retired breeders; mean ± SD body weight was 1.02 ± 0.10 kg. Guinea pigs used in the primary study were 8 to 11 weeks old; mean body weight was 0.74 ± 0.08 kg. Guinea pigs were purchased from a commercial vendor.4 All guinea pigs were considered to be healthy on the basis of results of a physical examination and a CBC. All procedures were approved by the Institutional Animal Care and Use Committee at the University of California-Davis.

Preliminary experiment

A vascular access port was surgically inserted in the right carotid artery of each of the 6 guinea pigs. All surgeries were performed by the same investigator (MJS) by use of a placement technique modified from a procedure described for rats.27 A 2F catheter4 was attached to each port. A commercial formulation of buprenorphine4 was administered. Guinea pigs were randomly assigned (by drawing numbers out of a container) into 1 of 3 groups (IM, IV, or OTM administration) at 1 of 2 doses (0.05 or 0.06 mg/kg). Each guinea pig received both doses by use of 2 routes of administration; there was a 7-day washout period between administrations. Blood samples were collected and assayed to measure buprenorphine concentrations. Several of the vascular access ports failed during the course of the preliminary experiment.

The pH of the oral cavity was measured by use of pH paper before OTM administration of buprenorphine. The oral cavity of each guinea pig then was cleaned (flushed with 3 to 6 mL of water followed by wiping the inside of the mouth with cotton-tipped applicators). The oral cavity was visually inspected to ensure there was no or only minimal food material within the oral cavity before OTM administration of buprenorphine.

On the basis of results for the preliminary experiment, 2 aspects were revised for the primary study. Because the vascular access ports were not reliable over time, guinea pigs in which a catheter had been placed in the right carotid artery were purchased from the commercial laboratory vendor.4 The doses of buprenorphine used did not result in a target plasma concentration of 1 ng/mL at any time. Therefore, the volume of distribution determined from the preliminary experiment was used to calculate a dose (0.2 mg/kg) with a higher likelihood of providing a target plasma concentration of 1 ng/mL.

Primary experiment

Eight guinea pigs, each with a 2F catheter surgically implanted in the right carotid artery, were purchased for use in the study. Aseptic technique was used during all catheter maintenance procedures and blood sample collections. Briefly, a wound clip (which held the catheter in place) was removed from the skin of the interscapular region. Catheter tubing (3 to 4 cm) was exteriorized from the subcutaneous space, and padded hemostats were used to occlude the catheter before removal of a metal stopper from the end of the catheter. The metal stopper was placed in 0.05% chlorhexidine solution4 during blood sample collection; it was rinsed with sterile saline (0.9% NaCl) solution prior to reinsertion in the catheter. A 23-gauge blunt-ended needle4 was inserted into the lumen of the catheter, and the blood sample was collected by use of a 3-syringe method (removal of heparin and blood, collection of the sample [0.2 to 0.3 mL], and reinjection of the heparin and blood in the first syringe to the guinea pig followed by administration of a heparin flush). Heparinized saline solution (4 U/mL) was used for blood collection and flushing of catheters to maintain patency between blood collections. When no samples were to be collected within 8 hours, the catheter was infused with a solution consisting of a mixture of heparin (500 U/mL) and 50% dextrose.5 After the catheter was flushed...
(heparinized saline solution or heparin-dextrose solution), the blunt-ended needle was removed, and the metal stopper was reinserted into the catheter. The padded hemostats were then removed, and the catheter was wiped with 0.05% chlorhexidine solution. The catheter was replaced back into the subcutaneous space, and the wound clip then was reattached.

A crossover experiment was conducted. Guinea pigs were randomly assigned (by drawing numbers out of a container) into 1 of 2 groups (IV or OTM) for the first administration of buprenorphine (0.2 mg/kg). For IV administration, guinea pigs were anesthetized with isoflurane (2% to 3%) in oxygen administered via face mask to prevent movement during drug administration. Isoflurane was discontinued immediately after buprenorphine administration (each guinea pig received isoflurane for <4 minutes). Blood samples were collected into heparinized tubes immediately before (time 0) and 1.5, 5, 10, 15, 30, 60, 120, 360, 720, 960, and 1,440 minutes after buprenorphine administration. There was a washout period of 7 days, and buprenorphine was then administered by the other route of administration.

The blood removed from each guinea pig over the course of the study was calculated to be <10% of each animal’s total blood volume. Collected blood samples were stored on ice until centrifugation (3,000 × g for 10 minutes); all samples were centrifuged within 1 hour after collection. Plasma was harvested and stored in cryotubes at −80°C until analysis; all samples were analyzed within 14 days after collection.

**pH of the oral cavity**

The same procedures described for the preliminary experiment were used for the primary experiment. The pH of the oral cavity was measured with pH paper, the oral cavity of each guinea pig was cleaned, and the oral cavity was visually inspected to ensure there was no or only minimal food material within the oral cavity before OTM administration of buprenorphine.

**Sedation score**

A sedation scale for guinea pigs was developed by modifying methods described for studies performed with rats and rabbits (Appendix). Sedation scores were assigned to each guinea pig immediately before handling for sample collection and at various time points after buprenorphine administration; investigators were aware of the route of administration for each guinea pig. Sedation was not scored in guinea pigs receiving buprenorphine via the IV route as well as 1 guinea pig receiving buprenorphine via the OTM route until 30 minutes after administration in an attempt to avoid possible confounding effects of isoflurane administration. The 1 guinea pig receiving buprenorphine via the OTM route was initially anesthetized with isoflurane with the intent that buprenorphine would be administered IV; however, we were unable to gain vascular access, and the administration route was switched to OTM.

**Analysis of plasma samples**

All plasma samples (preliminary experiment and primary experiment) were analyzed in an identical manner. Buprenorphine was quantified in guinea pig plasma with liquid chromatography–tandem mass spectrometry of protein-precipitated samples by use of a previously published method. Partial validation was performed, with guinea pig plasma used as the matrix. The response for buprenorphine was linear (R², 0.99). Recovery of buprenorphine from plasma was 88%. Precision and accuracy of the assay were determined by assaying buprenorphine quality control samples in replicates (n = 6). Accuracy (percentage of the nominal concentration) was 110% and 99% (preliminary experiment) and 109% and 95% (primary experiment) for 0.30 and 40 ng/mL, respectively. Precision (percentage relative SD) was 6.0% and 3.0% (preliminary experiment) and 3.0% and 5.0% (primary experiment) for 0.30 and 40 ng/mL, respectively. The assay was optimized to provide a limit of quantitation of 0.05 ng/mL and limit of detection of 0.025 ng/mL.

**Pharmacokinetic analysis**

Pharmacokinetic analysis was performed on plasma buprenorphine concentrations by use of noncompartmental analysis with a commercially available software program. Values for Cmax and Tmax following OTM administration were obtained directly from the plasma concentration data. Calculated pharmacokinetic parameters included λz, terminal-phase half-life, AUC from time 0 to infinity, and percentage of the AUC that was extrapolated. The terminal-phase half-life was calculated as 0.693/λz, and AUC was calculated by use of the log-linear trapezoidal method.

Statistical analyses were performed to assess differences in the half-life between IV and OTM administration. Data were analyzed by use of a nonparametric test (Wilcoxon signed rank test). Significance was set at values of P < 0.05. Time points at which plasma concentration of buprenorphine was higher than the target plasma concentration (1 ng/mL) were identified.

**Results**

**Preliminary experiment**

All guinea pigs remained healthy with no clinically apparent adverse effects of drug administration throughout the experimental period. Plasma concentrations of buprenorphine were <1 ng/mL, which is the minimum plasma concentration reported to be effective in some other species. All guinea pigs had a sedation score of 0 after buprenorphine administration. The pH of the oral cavity was 8 to 9 in each guinea pig prior to OTM administration of buprenorphine.
Primary experiment

All guinea pigs remained healthy with no clinically apparent adverse effects of drug administration during the study period. Plasma concentration of buprenorphine-versus-time curves for IV and OTM administration of buprenorphine at a dose of 0.2 mg/kg were plotted (Figure 1). Pharmacokinetic parameters were calculated for both routes of buprenorphine administration (Table 1). The mean C\text{max} after OTM administration was 2.4 ng/mL, and the mean Tmax was 71.2 minutes. The AUC from time 0 to infinity was 2,038.1 and 579.1 min\cdot ng/mL for IV and OTM administration, respectively. The terminal phase half-life was 184.9 and 173.0 minutes for the IV and OTM routes, respectively, and did not differ significantly \((P = 0.52)\) between the routes of administration. Norbuprenorphine was not detected in any of the samples, but buprenorphine glucuronide was detected.

Sedation scores were determined for both routes of administration (Table 2). One consistent finding for mildly sedated (sedation score = 1) guinea pigs was a flattened appearance to the caudal aspect of the dorsum and drooping of the ears (Figure 2). This portion of the back would become more rounded when the guinea pig was stimulated, and the ears would become more raised when guinea pigs were no longer sedate. Sedation scores at all time points for all guinea pigs, except for 2 guinea pigs, were 0 or 1. Those guinea pigs were moderately sedated when first scored at 30 minutes and less sedate when scored at 60 minutes after IV administration of buprenorphine. At 360 minutes, no sedation was evident (sedation score, 0) for any of the guinea pigs. No guinea pigs regurgitated or had signs of nausea after buprenorphine treatment.

Oral pH was 8 to 9 in each guinea pig prior to OTM administration of buprenorphine. Guinea pigs did not object to OTM administration, and salivation was not observed.
both the carotid artery and medial saphenous vein, samples differed from those for samples obtained from peripheral veins. As was reported for cats.

Drug profiles after OTM administration of buprenorphine glucuronide or as buprenorphine glucuronide. Elimination buprenorphine primarily as the parent compound or as buprenorphine glucuronide.

The bioavailability of buprenorphine from the administration site via swallowing, which would make the drug susceptible to gastrointestinal effects or hepatic first-pass effects. This ultimately would have led to lower systemic drug concentrations. The relatively large volume (0.2 mg/kg, IV or OTM).

The highest sedation score observed for the guinea pigs of the present study was 2 (scale, 0 to 3). Seven of 8 guinea pigs had a sedation score of 0 until 30 minutes after OTM administration of buprenorphine. At that time, 2 of 8 guinea pigs had a sedation score of 1, which was maintained through 120 minutes. By 360 minutes after OTM administration, all guinea pigs again had a sedation score of 0. This may have reflected drug absorption over time. In comparison, when guinea pigs received isoflurane for IV administration of buprenorphine, sedation was not scored until 30 minutes after administration of buprenorphine to avoid confounding effects of the inhalation anesthetic. At 30 minutes after IV administration, 2 of 8 guinea pigs had a sedation score of 2, whereas 4 of 8 had a sedation score of 1. These scores decreased over time, and by 60 minutes, 4 of 8 guinea pigs had a sedation score of 1 and no guinea pigs had a sedation score of 2. By 120 minutes after IV administration, 2 of 8 guinea pigs had a sedation score of 1, and all guinea pigs had a sedation score of 0 by 360 minutes after IV administration. This gradual decrease in sedation over time may have been a reflection of buprenorphine metabolism or may have been attributable to the animals recovering from the effects of isoflurane. Regardless of the administration route, no guinea pig had a sedation score > 1 after 30 minutes, and no sedation was evident after 120 minutes. This has clinical implications because sedative effects can challenge a clinician’s ability to accurately determine the comfort level of a patient. Additionally, all guinea pigs in the primary experiment reported here were young and healthy; thus, they may have been less likely to be sedated than a geriatric, diseased, or postsurgical guinea pig. Anecdotally, the authors have detected a substantial degree of sedation.

Discussion

Bioavailability of a drug is the fraction of an administered dose that is absorbed and reaches the systemic circulation intact. For the purpose of the study reported here, bioavailability was used to represent the fraction of the dose absorbed. The physiochemical properties of buprenorphine, along with the relative alkaline pH of the oral cavity of guinea pigs, would predict that buprenorphine would be well absorbed after OTM administration (similar to the situation for cats, which have an oral pH almost identical to that of guinea pigs). In the present study, the mean bioavailability of buprenorphine after OTM administration to guinea pigs was 28.4% as determined by use of samples obtained from the carotid artery. The low bioavailability in the present study may have been attributable to loss of drug from the administration site via swallowing, which would make the drug susceptible to gastrointestinal effects or hepatic first-pass effects. This ultimately would have led to lower systemic drug concentrations. The relatively large volume (> 0.5 mL for most guinea pigs) for OTM administration in addition to the small buccal cavity of guinea pigs caused the animals to swallow rapidly, despite the fact the drug was administered slowly and against the buccal mucosa. Administration of a more concentrated solution may be useful for achieving target concentrations, especially if part of the dose is swallowed.

In many species, buprenorphine is eliminated primarily as the metabolite norbuprenorphine. In contrast to results reported for other species, norbuprenorphine was not detected in the guinea pigs of the present study. Instead, guinea pigs appeared to eliminate buprenorphine primarily as the parent compound or as buprenorphine glucuronide.

Collection of blood samples from the carotid artery was used because of the potential impact of the site of blood sample collection on time-concentration drug profiles after OTM administration of buprenorphine, as was reported for cats. In that study, pharmacokinetic parameters for jugular venous blood samples differed from those for samples obtained from both the carotid artery and medial saphenous vein, including overestimation of Cmax and AUC and underestimation of clearance. Because blood that perfuses the buccal mucosa (the presumed site of drug absorption after OTM administration) flows into the jugular veins, the concentrations for samples collected from this site do not represent concentrations for blood samples obtained after mixing in the central compartment. On the basis of these findings, it is recommended that arterial blood samples be used in studies conducted to calculate estimates of bioavailability after OTM administration. Venous blood samples obtained from vessels in the pelvic limbs are an alternative to carotid artery blood samples for evaluation after OTM administration of medications; however, in guinea pigs, catheter placement and prolonged maintenance of catheter patency are likely to be challenging in a pelvic limb location. Additionally, it has been suggested that estimates derived from samples obtained from peripheral veins are not as meaningful as those obtained for arterial blood samples.

Figure 2.—Photograph of a guinea pig displaying a flattened caudal aspect of the dorsum and drooping of the ears in response to administration of buprenorphine (0.2 mg/kg, IV or OTM).
after OTM administration of buprenorphine at a dose of 0.2 mg/kg to diseased or postsurgical guinea pigs; this included substantial sedation during the postcastration period for one of the adult guinea pigs used in the preliminary experiment. However, that guinea pig did not have evidence of sedation during the preliminary experiment.

Limitations of the present study included the small sample size. Additionally, the population evaluated in the primary experiment was of a single sex and young age, which made it fairly homogenous. As a result, there may be differences in pharmacokinetic outcomes with other guinea pig strains or age groups or with female guinea pigs. The effects of short-term use of isoflurane for anesthesia on pharmacokinetics of buprenorphine in guinea pigs is unknown, and further studies to evaluate such effects in awake and anesthetized subjects will be necessary to determine whether isoflurane administration impacts the pharmacokinetic values. The authors were aware of the route of administration when performing sedation scoring, which may have affected the results. Additionally, the authors considered the sedation scoring system to be suboptimal because of the fact that it was based on scoring systems described for rats28 and rabbits,29 which may display signs of sedation in a different manner than guinea pigs do. One consistent finding for mildly sedated (sedation score = 1) guinea pigs was a flattened appearance of the caudal aspect of the dorsum. This portion of the back would become more rounded when the guinea pig was stimulated. In addition, drooping of the ears was a notable characteristic during sedation. These unique characteristics of guinea pigs during sedation may be a beneficial addition to future sedation scoring systems.

The pharmacokinetic values of the present study provided preliminary data necessary for future pharmacodynamic studies and further evaluation of the buprenorphine dose and duration of action as well as evaluation of other administration routes in guinea pigs. The primary experiment was a single-dose experiment with a large increase in dose from the preliminary experiment (0.2 vs 0.06 mg/kg, respectively). It is possible that a dose between these 2 doses may result in adequate plasma concentrations of buprenorphine and potentially decrease sedative effects in diseased or postsurgical guinea pigs. The SC route of administration also should be evaluated because that is a common route of administration used for guinea pigs.

Acknowledgments

Supported by the Association of Exotic Mammal Veterinarians and the Center for Companion Animal Health, School of Veterinary Medicine, University of California-Davis.


Footnotes

a. Access Technologies, Skokie, Ill.
b. Buprenex (0.3 mg/mL), Hospira Inc, Lake Forest, Ill.
c. Charles River Laboratories, Wilmington, Mass.
d. VetOne, Boise, Idaho.
e. SAI Infusion Technologies, Lake Villa, Ill.
g. Phoenix WinNonlin, version 6.2, Certara USA Inc, Princeton, NJ.

References


**Appendix**

Sedation scoring system used for 8 guinea pigs (*Cavia porcellus*) that received 1 dose of buprenorphine (0.2 mg/kg, IV and OTM) in a crossover experiment.

<table>
<thead>
<tr>
<th>Category</th>
<th>Sedation score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>No sedation</td>
<td>0</td>
<td>No abnormalities in posture, ambulation, jaw tone, palpebral reflex, or resistance to restraint and handling (struggles to escape and attempts to withdraw limbs).</td>
</tr>
<tr>
<td>Mild sedation</td>
<td>1</td>
<td>Stands normally but no ambulation, palpebral reflex is present, bottom lip may droop slightly, and moderate resistance to restraint and handling.</td>
</tr>
<tr>
<td>Moderate sedation</td>
<td>2</td>
<td>Sternal recumbency with variable head position, palpebral reflex may be absent, drooping of bottom lip, and minimal resistance to restraint and handling.</td>
</tr>
<tr>
<td>Heavy sedation</td>
<td>3</td>
<td>Lateral recumbency, may not move when stimulated, palpebral reflex is absent, drooping of bottom lip, and no or only slight resistance to restraint and handling.</td>
</tr>
</tbody>
</table>