Pharmacokinetics of buprenorphine following intravenous administration in dogs

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Objective—To determine pharmacokinetics of buprenorphine in dogs after IV administration.

Animals—6 healthy adult dogs.

Procedures—6 dogs received buprenorphine at 0.015 mg/kg, IV. Blood samples were collected at time 0 prior to drug administration and at 2, 5, 10, 15, 20, 30, 40, 60, 90, 120, 180, 240, 360, 540, 720, 1,080, and 1,440 minutes after drug administration. Serum buprenorphine concentrations were determined by use of double-antibody radioimmunoassay. Data were subjected to noncompartmental analysis with area under the time-concentration curve to infinity (AUC) and area under the first moment curve calculated to infinity by use of a log-linear trapezoidal model. Other kinetic variables included terminal rate constant (kₜ), elimination half-life (t½), plasma clearance (Cl), volume of distribution at steady state (Vdₛ), and mean residence time (MRT). Time to maximal concentration (Tₘₚₙₚₙ) and maximal serum concentration (Cₘₚₙₚₚ) were measured.

Results—Median (range) values for Tₘₚₚ and MRT were 2 minutes (2 to 5 minutes) and 264 minutes (199 to 600 minutes), respectively. Harmonic mean and pseudo SD for t½ were 270 ± 130 minutes; mean ± SD values for remaining pharmacokinetic variables were as follows: Cₘₚₚ, 14 ± 2.6 ng/mL; AUC, 3,082 ± 1,047 ng•min/mL; Vdₛ, 159 ± 0.285 L/kg; Cl, 5.4 ± 1.9 mL/min/kg; and, kₜ, 0.0026 ± 0.012.


Buprenorphine is a semisynthetic partial μ-opioid receptor agonist that appears to provide better analgesia for soft tissue pain than for major bone trauma and surgery.¹⁻³ Buprenorphine is widely used in small animal practices in Europe, Australia, and South Africa and is commonly used, particularly in cats, in the United States.⁴⁻⁶ Only 1 study⁷ has been conducted on the pharmacokinetics of buprenorphine in dogs, during which suprapharmacologic doses (0.7 to 2.6 mg/kg) were used, thus limiting applicability of these data to clinical use. Pharmacokinetics of buprenorphine at clinically relevant doses (0.005 to 0.02 mg/kg) in dogs⁸⁻¹⁰ has not been described. The purpose of the study reported here was to determine the pharmacokinetics of buprenorphine following IV administration in dogs and to establish a clinically relevant dosing regimen.

Materials and Methods

Animals—This study protocol was approved by the Cornell University Institutional Animal Care and Use Committee. Six adult dogs (2 male and 4 female) were studied, which included 2 Golden Retrievers, 2 mixed-breed dogs, 1 Labrador Retriever, and 1 Chesapeake Bay Retriever. No clinically important abnormalities were found on physical examination, CBC determination, and serum biochemical analysis. Mean ± SD age and body weight of dogs was 27 ± 10 months and 31 ± 6.8 kg, respectively.

ABBREVIATIONS

<table>
<thead>
<tr>
<th>ABBREVIATION</th>
<th>DESCRIPTION</th>
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<tbody>
<tr>
<td>AUC</td>
<td>Area under the time-concentration curve to infinity</td>
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<td>AUMC</td>
<td>Area under the first moment curve</td>
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<td>Cl</td>
<td>Plasma clearance</td>
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<td>Cₘₚₚ</td>
<td>Maximal serum drug concentration</td>
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<td>E₅₀</td>
<td>Overall body extraction ratio</td>
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<tr>
<td>kₜ</td>
<td>Rate constant associated with the terminal elimination phase</td>
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<tr>
<td>MRT</td>
<td>Mean residence time</td>
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<tr>
<td>t½</td>
<td>Elimination half-life</td>
</tr>
<tr>
<td>Tₘₚₚₚ</td>
<td>Time of maximal serum drug concentration</td>
</tr>
<tr>
<td>Vdₛ</td>
<td>Volume of distribution at steady state</td>
</tr>
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Received August 9, 2007.
Accepted October 29, 2007.

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Supported by the Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, and by the Barry and Savannah French-Poodle Memorial Fund.


The authors thank Professor Hollis N. Erb for assistance with the statistical analysis. Address correspondence to Dr. Krotscheck.
Food was withheld from all dogs for ≥ 8 hours. One jugular furrow was clipped and a topical anesthetic agent applied. After 30 minutes, dogs were manually restrained; the jugular furrow aseptically prepared; and a 19-gauge, 30.5-cm (12-in) jugular catheter aseptically inserted in a caudal direction until it was estimated, based on prior measurement, to be positioned within the cranial vena cava. The catheter was secured with tape and a soft padded wrap. This catheter was only used for blood sample collection.

Drug administration and data collection—Buprenorphine HCl (0.015 mg/kg, IV bolus) was administered into a cephalic vein over 5 seconds. Clinical signs typical of opioid administration (eg, vomiting, swallowing, retching, ptalism, sedation, and recumbency) were recorded at each blood sample collection time. Lengths of clinically apparent effects were recorded based on each dog’s recumbency, appropriate response to stimuli, and ability to ambulate normally.

Blood sample collection and handling—Blood samples (3 mL) were collected into evacuated tubes at time 0 prior to drug administration and at 2, 5, 10, 15, 20, 30, 40, 60, 90, 120, 180, 240, 360, 540, 720, 1,080, and 1,440 minutes after drug administration. A standard 3-syringe technique was used to withdraw blood (3 mL) from the jugular catheter to ensure the samples were not diluted. The volume of blood withdrawn was replaced with an equal volume of saline (0.9% NaCl) solution at each collection. All samples were processed within 2 hours after collection. The blood was allowed to clot and then centrifuged for 15 minutes at 560 X g. The serum was collected, transferred into polypropylene serum transport tubes, and frozen at –70°C until overnight shipment on dry ice to the analytical laboratory.

Serum sample analysis—Serum samples were analyzed by use of a commercial sodium iodide 1125 radioimmunoassay kit for the quantitative measurement of buprenorphine. The lower limit of detection delineated by the manufacturer is 0.1 ng/mL, and the kit has extensive cross-reactivity with the N-dealkylated metabolite of buprenorphine. In humans, these metabolites are undetectable after a single dose (0.3 mg, IV) or following constant rate infusions ranging from 32 to 239 µg/h. The assay was validated for canine serum by comparing the predicted to known concentrations of control samples based on a standard curve. The standard curve was generated by the addition of known amounts of each drug to canine serum. The lower and upper limits of quantification were 0.2 ng/mL and 8.5 ng/mL, respectively. The coefficient of variation for 3 control samples that spanned the detection range was < 15% for the high-concentration control and ≤ 20% for the low-concentration control. All samples (validation and study) were analyzed in duplicate; the coefficient of variability was based on ≥ 5 sets of control samples.

Pharmacokinetic analysis—For each dog, drug concentration versus time data were subjected to noncompartmental analysis with AUC and AUMC calculated to infinity by use of a log-linear trapezoidal model. A minimum of 3 data points were used to calculate the k el and the terminal t ½ according to the following formula:

\[ t_{1/2} = \frac{0.693}{k_{el}} \]

From these values, other kinetic variables were calculated as follows:

\[ C_{max} = \text{peak concentration} \]

\[ E_{body} = \frac{\text{body clearance}}{\text{cardiac output}} \]

Cardiac output was determined for each dog by use of the following formula:

\[ \text{Cardiac output} = 180 \times \text{body weight (kg)}^{0.19} \]

All pharmacokinetic data were tested for normality by use of a normal probability plot and the Shapiro-Wilk statistic (with P ≤ 0.10 used to indicate nonnormality). The harmonic mean and pseudo SD were calculated for t ½. Mean ± SD or median (range) was calculated for the remaining variables.

Results

Clinical effects—Dogs did not react adversely to jugular catheter insertion, and all catheters remained patent for the duration of the study. No dog had behaviors of swallowing, retching, vomiting, or ptalism. All dogs had signs of sedation for a median (range) duration of 3.5 hours (1.5 to 4 hours) with onset 5 minutes after IV administration in 6 dogs.

Figure 1—Plasma concentrations of buprenorphine (mean ± SD) after IV administration in 6 dogs.
after IV injection of the drug. Four of 6 dogs preferred lateral recumbency during this time; the other 2 preferred sternal or standing positions. Five of 6 dogs were assessed to be clinically normal 6 hours after injection; the sixth dog was difficult to evaluate completely because of its nervous nature. All dogs were able to ambulate without ataxia at this time.

**Pharmacokinetics**—All data, except $T_{\text{max}}$ and MRT, were normally distributed and were reported as mean ± SD values; $T_{\text{max}}$ and MRT were reported as median (range) values. The $C_{\text{max}}$ achieved in the 6 dogs was $14 ± 2.6$ ng/mL; $\tau/2$ achieved was $270 ± 130$ minutes (Table 1). The curvilinear (mean ± SD) concentration-time plot was determined (Figure 1).

### Table 1—Pharmacokinetic variables for each of the 6 dogs and the summarized data.

<table>
<thead>
<tr>
<th>Dog</th>
<th>$T_{\text{max}}$ (min)</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>AUC (ng•min/mL)</th>
<th>$\tau/2$ (min)</th>
<th>$V_d$ (L/kg)</th>
<th>CI (mL/min/kg)</th>
<th>MRT (min)</th>
<th>$k_{\text{e}}$ (min$^{-1}$)</th>
<th>$E_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>16</td>
<td>2,783</td>
<td>279</td>
<td>1.26</td>
<td>5.4</td>
<td>234</td>
<td>0.0025</td>
<td>0.059</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>17</td>
<td>4,222</td>
<td>292</td>
<td>1.78</td>
<td>3.6</td>
<td>499</td>
<td>0.0024</td>
<td>0.036</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>11</td>
<td>1,804</td>
<td>175</td>
<td>1.66</td>
<td>8.3</td>
<td>199</td>
<td>0.004</td>
<td>0.084</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>12</td>
<td>2,302</td>
<td>202</td>
<td>1.34</td>
<td>6.5</td>
<td>238</td>
<td>0.002</td>
<td>0.079</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>13</td>
<td>2,945</td>
<td>454</td>
<td>1.49</td>
<td>5.1</td>
<td>293</td>
<td>0.0015</td>
<td>0.054</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>17</td>
<td>4,439</td>
<td>645</td>
<td>2.03</td>
<td>3.4</td>
<td>600</td>
<td>0.0011</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Mean: $2.5 ± 1.2$; Median: $2.6 ± 1.048$; Range: 2–5

*Harmonic mean and pseudo SD. – Not applicable.

**Discussion**

Buprenorphine is a highly lipophilic derivative of the morphine alkaloid thebaine and is a member of the 6,14-endo-ethanotetrahydro-oripavine class. The median lethal dose of buprenorphine HCl following IV administration in dogs is 79 mg/kg, and in rats the therapeutic index (median lethal dose/median effective dose) is 12,313.

Traditionally, buprenorphine has been thought of as a partial $\mu$-opioid receptor agonist and a $\kappa$-receptor antagonist. Results of a more recent study indicate that, with regard to its antinociceptive effect, buprenorphine acts as a full receptor agonist at $\mu$-receptors in rats. The $\mu$-effects are responsible for supraspinal analgesia, respiratory depression, and miosis. Buprenorphine might have a wider safety profile, compared with other full $\mu$-receptor agonists, especially regarding respiratory depression. Properties of $\kappa$-antagonism are some degree of spinal analgesia, dysphoria, and psychomimetic effects. Buprenorphine also binds to the $\delta$-opioid receptor but with lower affinity than to either the $\mu$- or $\kappa$-receptors. The functional importance of this is unknown. As an avid binder to the $\mu$-opioid receptor, buprenorphine might displace other $\mu$-receptor agonists given concurrently. As such, it could reverse some of the adverse effects of pure receptor agonists while retaining some analgesia. An additional opioid receptor (the ORL 1 receptor) has been identified. Nociceptin, also referred to as orphanin, is this receptor’s natural ligand. Nociceptin acts to substantially decrease dopamine concentrations, either directly as an endogenous antagonist of dopamine transport or by inhibiting $\gamma$-aminobutyric acid. Within the CNS, actions of nociceptin are either similar or opposite to those of opioids, depending on their location, Buprenorphine reportedly is a partial receptor agonist at the ORL 1 receptors, whereas its metabolite norbuprenorphine is a full receptor agonist at these receptors, although this is controversial.

Pharmacokinetics of buprenorphine have been evaluated in multiple species. In the summation of previously published data, broad similarities were found between the disposition of morphine, buprenorphine, and pethidine in dogs and cats. Following IV administration of buprenorphine at 0.01 mg/kg in cats and of 0.7 mg/kg to 4.8 mg/kg in dogs, $\tau/2$ was $417$ minutes and 1,257 minutes, $V_d$ was $7.1$ L/kg and 5.1 L/kg, and CI was $17.7$ mL/kg/min and 10.5 mL/kg/min for cats and dogs, respectively. Other than the large differences in doses, several other important differences also were found in these studies. Measurement of buprenorphine concentrations in the study in cats were made by use of the same double-antibody radioimmunoassay used in our study presented here, whereas the original study dogs used high-performance liquid chromatography. Use of the radioimmunoassay does not allow for discrimination between buprenorphine and its metabolites, whereas use of high-performance liquid chromatography does. Buprenorphine is metabolized in the liver by N-dealkylation and glucuronidation, resulting in norbuprenorphine and buprenorphine-3-glucuronide, respectively. In dogs, the highest concentrations of buprenorphine-3-glucuronide are detected within 2 minutes of IV administration of a bolus and the decay of buprenorphine and buprenorphine-3-glucuronide parallel each other in plasma. The formation of norbuprenorphine appears to be negligible. When buprenorphine-3-glucuronide is administered directly into the duodenum, enterohepatic recirculation does occur, but the lack of free buprenorphine in the plasma indicates that any recirculation of the primary metabolite does not affect the half-life of the drug. The metabolite profiles following parenteral administration of buprenorphine are similar in humans and dogs, indicating that in dogs, as in humans, the impact of cross-reaction with the metabolite is likely to be minimal after a single dose. Despite binding of norbuprenorphine to the radioimmunoassay antibody, results of a study in which a radiotracer was used indicate that little norbuprenorphine is found after a single
In cats, the metabolic products of buprenorphine are unknown but their ability for glucuronidation is limited.28 In humans, serum drug concentrations do not change in proportion to dose, nor do any subjective and physiologic measurements29 suggesting that the disposition of buprenorphine cannot be predicted by dose. As such, clinical use should be based on a scientific study that describes the relationship.

The Cmax of buprenorphine in dogs in the study presented here was 14 ± 2.6 ng/mL, which is similar to values of 15.5 ng/mL (7.1 to 26.7 ng/mL) and 12.5 ng/mL (2.55 to 19.4 ng/mL) reported27,28 for cats after IV administration of buprenorphine at 0.01 mg/kg and 0.02 mg/kg, respectively. In our study, Tmax was achieved at the first blood sample collection time (2 minutes) following IV administration of buprenorphine in 5 of 6 dogs. It is a limitation of this study that the first blood sample collection time following IV administration of buprenorphine was at 2 minutes because higher and earlier peaks of Cmax and Tmax may have been missed. Because the purpose of this study was to describe the pharmacokinetics of buprenorphine at a clinically used dose, a higher or earlier Cmax or Tmax are unlikely to make a clinical difference. The AUC, representing the total time course of the drug in the body, was 3,082 ng•min/mL in our study, which is higher than values of AUC previously reported for cats (722 to 2,712 ng•min/mL).27,28 Drug administration was IV in each study, thus removing the influence of absorption following oral administration on AUC. As such, differences between dogs and cats are likely the result of differences in Cl, with Cl being less in dogs. The τ/2 of 270 minutes for dogs in our study is similar to that found for cats (368 and 417 minutes).27,28

In a previous study, the disposition of buprenorphine has been reported for dogs at doses that range from 0.7 to 2.6 mg/kg.7 Substantial differences exist among the disposition values between our study and the earlier study.7 However, care must be taken when comparing data of these 2 studies because of differences in study designs. Some of these differences reflect the reported variable (eg, the AUC is not reported for the different doses in the previous study7). In addition, the standard curve of Garrett and Chandran7 did not span the concentrations of drug detected, control samples were not diluted, and evidence of the lower limit of quantitation was not provided. Also, blood samples were collected from the same catheter into which the drug was administered. As such, failure to remove all drug can cause inaccuracies in measurements of plasma drug concentrations (overestimated) and volume of distribution (underestimated).

The volume of drug cleared per unit time is independent of dose for drugs that follow first-order kinetics.30 However, a recent study in rats reported nonlinear kinetics (ie, zero-order elimination) of buprenorphine, likely indicating nonlinear Cl.26 The free fraction of the drug in plasma is determined by the free concentration of the drug. Therefore, in drugs having nonlinear kinetics, the free fraction is not consistent over a range of concentrations.31 The Ebody is directly related to the properties of the drug. The Ebody is the percentage of the drug cleared by the body during a single passage through the clearing organs. Mean ± SD value of Ebody for our study was 0.057 ± 0.019, indicating a low extraction ratio. For drugs with low extraction ratios, the total body clearance is proportional to the free fraction of the drug in plasma, whereas Cl of the free drug is independent of the free fraction of the drug in plasma. Higher doses of buprenorphine therefore may have a higher free fraction of buprenorphine, resulting in a higher total body clearance.26

The Vd in this study was less than previously reported for dogs7 or cats.27,28 A high Vd indicates that the drug is not retained in the plasma, as might occur for drugs distributed to fat, or bound to or sequestered in peripheral tissues. Differences may reflect catheter use but also may reflect dose dependency. Protein-binding of highly protein-bound drugs such as buprenorphine (96%) may become saturated at higher doses.26 This increase is consistent with the phenomenon of concentration-dependent binding to plasma proteins; a larger fraction of the drug is shifted to other tissue compartments from the plasma compartment, which could potentially explain the higher volume of distribution with higher doses.26

Pharmacokinetic data are not the major determining factor of dosing regimens for buprenorphine in dogs. Rather, regimens are based on antinociceptive findings by use of a tooth pulp stimulation technique. In a study32 in dogs,33 on subcutaneous and sublingual administration of buprenorphine (0.02 to 0.04 mg/kg), the AUC, antinociceptive plasma concentrations in dogs and cats are similar for fentanyl at 0.93 and 1.07 ng/mL, respectively.33,34 Assuming pharmacodynamic responses are similar between dogs and cats for buprenorphine, based on pharmacokinetics described in our study, IV administration of buprenorphine may provide analgesia for 3 to 4 hours. Added to this time would be effects that are maintained as a result of slow dissociation between ligand-receptor affinity or biophase equilibrium.

The wide range of τ/2 values in our study may reflect differences in the extent of redistribution from peripheral tissues or the speed of clearance or cytochrome P450 polymorphism. The terminal half-life after IV administration is the time required for the blood or plasma drug concentration to decrease by 50% after pseudo-equilibrium is complete, differences in clearance (ie, the ability of the body to eliminate the drug) are a possible reason for the variability in τ/2 in our study. Alternatively, the cytochrome P450 system, which is responsible for metabolizing buprenorphine, has been
determined to have interethnic variability in expression in humans. The implications of this are that standard doses of a drug, when metabolized by such a polymorphic enzyme system, could result in prolonged therapeutic effects, lack of effect, or drug toxicity. If this is true for dogs, it could explain the variability in $t_{1/2}$. On the other hand, because our study population consisted of mainly retriever-type dogs, we may have limited the effect of cytochrome P450 variability in drug metabolism. Assuming a direct relationship between antinociceptive effects and plasma drug concentrations, the almost 4-fold variation in $t_{1/2}$ (174 to 645 minutes) in individual dogs of our study supports the importance of dosing regimens being designed around patient response rather than predefined dosing intervals.

The long duration of action of buprenorphine as an analgesic has been ascribed to its slow dissociation from the µ-opioid receptor, resulting in fewer clinical signs of opioid withdrawal and making it an ideal drug for the management of opioid dependence. In 2005, Yassen et al. found that the slow onset and offset of the antinociceptive effect of buprenorphine in humans are not caused by slow receptor association-dissociation kinetics but rather by slow biophase equilibration kinetics. Biophase equilibration determines the disposition characteristics of an opioid at the effect site and influences the biological effect intensity and the duration of the antinociceptive effect. Because of its high lipophilicity, buprenorphine is assumed to readily cross the blood-brain barrier, with the delay in onset of the antinociceptive effect most likely caused by distribution of the drug within the brain parenchyma itself. The decline of buprenorphine concentration from the brain of rats is markedly slower than from plasma ($t_{1/2}$ brain = 2.3 hours vs $t_{1/2}$ plasma = 1.4 hours). The brain-to-plasma concentration ratio after a single dose of buprenorphine administered IV is 3.0 at 15 minutes and 10.5 at 6 hours after administration. This has been confirmed in baboons, which also eliminate buprenorphine slower from the brain than from plasma. These findings strongly suggest that the elimination of buprenorphine from the brain is the rate-limiting step in termination of the drug's action. In cats, a considerable lag time exists between the peak drug concentration and the dynamic effect, likely the result of biophase equilibration. The thermal threshold increased between 30 and 360 minutes after IV administration of 0.02 mg/kg transmucosally, with the maximal effect occurring at 90 minutes, whereas $T_{\text{max}}$ occurred at 1 to 2 minutes after IV administration.

The study reported here represents the first in vivo study in which pharmacokinetics of buprenorphine were studied and described at a clinically relevant dose in dogs. Further antinociceptive testing is required to determine the therapeutically effective drug concentration and when it is achieved.

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f. Double antibody, Diagnostics Products Corp, Los Angeles, Calif.