Bioavailability and pharmacokinetics of oral and injectable formulations of methadone after intravenous, oral, and intragastric administration in horses

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Objective—To characterize the bioavailability and pharmacokinetics of oral and injectable formulations of methadone after IV, oral, and intragastric administration in horses.

Animals—6 healthy adult horses.

Procedures—Horses received single doses (each 0.15 mg/kg) of an oral formulation of methadone hydrochloride orally or intragastrically or an injectable formulation of the drug orally, intragastrically, or IV (5 experimental treatments/horse; 2-week washout period between each experimental treatment). A blood sample was collected from each horse before and at predetermined time points over a 360-minute period after each administration of the drug to determine serum drug concentration by use of gas chromatography–mass spectrometry analysis and to estimate pharmacokinetic parameters by use of a noncompartmental model. Horses were monitored for adverse effects.

Results—In treated horses, serum methadone concentrations were equivalent to or higher than the effective concentration range reported for humans, without induction of adverse effects. Oral pharmacokinetics in horses included a short half-life (approx 1 hour), high total body clearance corrected for bioavailability (5 to 8 mL/min/kg), and small apparent volume of distribution corrected for bioavailability (0.6 to 0.9 L/kg). The bioavailability of methadone administered orally was approximately 3 times that associated with intragastric administration.

Conclusions and Clinical Relevance—Absorption of methadone in the small intestine in horses appeared to be limited owing to the low bioavailability after intragastric administration. Better understanding of drug disposition, including absorption, could lead to a more appropriate choice of administration route that would enhance analgesia and minimize adverse effects in horses. (Am J Vet Res 2012;73:290–295)

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt;</td>
<td>Area under the concentration-time curve from 0 minutes to the last time point</td>
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<tr>
<td>CI</td>
<td>Total body clearance</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximum serum drug concentration</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>F</td>
<td>Bioavailability</td>
</tr>
<tr>
<td>MRT&lt;sub&gt;0-∞&lt;/sub&gt;</td>
<td>Mean residence time extrapolated to infinity</td>
</tr>
<tr>
<td>pKa</td>
<td>Acid-base dissociation constant</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>Half-life</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Time to maximum concentration</td>
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<tr>
<td>V&lt;sub&gt;d&lt;/sub&gt;</td>
<td>Apparent volume of distribution</td>
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In general, opioids are effective analgesic drugs used to treat acute and chronic pain in humans and other animals. However, opioids have limited use in equine medicine due to severe adverse effects, such as sympathetic stimulation and CNS excitation, increase of locomotor activity, and decrease of gastrointestinal tract motility, which are particularly notable after IV administration. Therefore, selection of alternative routes for opioid administration may be a means to achieve satisfactory therapeutic effects with minimal adverse effects in horses. Oral administration is probably the easiest and most convenient and cost-effective route for drug delivery and may be associated with fewer opioid-induced adverse effects. However, the oral disposition, including absorption, of opioid drugs is variable in horses due to factors...
that are known to affect the F of the drugs, such as drug properties, gastrointestinal and drug pH, physiologic characteristics of the gastrointestinal tract, and genetic factors (eg, transporter proteins).7

Methadone is an effective opioid agent that has unique properties and is used to treat severe acute and chronic, neuropathic, and cancer-related pain in humans.8–11 Inter- and intraindividual variability in the disposition of methadone after oral administration in humans have been described. Although methadone has physicochemical characteristics favorable for good absorption, its oral F in humans has been reported to be 30% to 80%.12 Methadone pharmacokinetics in people are characterized by rapid absorption, widespread tissue distribution, and long elimination t1/2.12–14 In human hepatocytes and enterocytes, methadone is extensively metabolized (via N-demethylation) to inactive metabolites by the enzymes in the CYP superfamily, primarily CYP3A4 and, to a lesser extent, CYP2D6.8,10,15–17 In addition, CYP2B has been reported to be the primary metabolizing enzyme in humans.18 However, in dogs, methadone has poor oral F, short elimination t1/2, and rapid Cl. Specific metabolic enzymes and metabolites are still not fully characterized.19,20

Because it is a synthetic μ-opioid receptor agonist and an N-methyl-D-aspartate receptor antagonist, methadone is usually administered as a racemic mixture of levo (l)- and dextro (d)-isomers. A commercial preparation of methadone was recently investigated in horses after single oral administration.21 Concentrations of methadone greater than the effective or therapeutic concentration reported for humans (33 to 59 ng/mL) were measured in the serum of horses, and no adverse effects were observed. The oral pharmacokinetics of methadone in horses are characterized by a short elimination t1/2, rapid Cl, and small V.21 However, the F of this drug was not estimated in that study and the process of methadone absorption after oral administration in horses needs further investigation.

Methadone is a highly lipophilic drug that has physicochemical characteristics related to high solubility and permeability; these characteristics favor oral or gastrointestinal drug absorption, which suggests that oral administration of this drug is appropriate.22,23 In horses, oral administration of methadone could potentially benefit pain management by limiting typical opioid-induced adverse effects such as excitation and gastrointestinal tract stasis that develop following IV administration. However, drug absorption and oral disposition of methadone in horses are still not completely described. The purpose of the study reported here was to characterize the F and pharmacokinetics of oral and injectable formulations of methadone in horses, oral, and intragastric administration in horses. The intent was to assess oral and intestinal mucosa absorption, thereby providing better understanding of the absorption process of methadone in horses.

Materials and Methods

Animals—Six healthy adult horses (5 Thoroughbreds [3 geldings and 2 females] and 1 Quarter Horse [a gelding]) were used in the study. For this group of horses, mean ± SD body weight was 504.6 ± 39.37 kg and mean age was 5.5 ± 1.87 years. As a selection criterion, horses had not received any medication for at least 4 weeks prior to the study. Horses were placed in stalls for acclimation 2 days before each experiment and had free access to pasture during the washout period between experiments. Weight was determined before each experiment for drug dose calculation. Horses received a complete pelleted ration twice daily, and food was withheld for 12 hours prior to drug administration. They were again fed 6 hours after drug administration. Horses had free access to water during the entire study period. From the first to the end of the last experiment, a 14-gauge catheter was maintained in the left jugular vein of each horse for blood sample collection. This study was approved by the Louisiana State University Institutional Animal Care and Use Committee.

Study design—In a randomized crossover design, each horse received each of 5 experimental treatments once; a 2-week washout period was allowed between each experimental treatment. The experimental treatments comprised a single dose (0.15 mg/kg) of an oral or injectable formulation of methadone hydrochloride via oral administration (ie, drug solution was directly squirted into the mouth by use of a syringe) or via intragastric administration through a nasogastric tube (following dose administration, the tube was immediately flushed with distilled water [volume of water = 2 X tube’s capacity]). Each horse also received a single dose (0.15 mg/kg) of the injectable formulation of methadone via IV administration; this experimental treatment was administered via venipuncture in the jugular vein that was not used for blood sample collection.

Horses were monitored during the study for possible adverse effects. Heart and respiratory rates (assessed via auscultation and counting respiratory movements, respectively) were recorded; behavior, excitation or sedation (low head position), and locomotor activity were observed and noted. All horses were returned to the herd after conclusion of the study.

Blood sample collection and clinical evaluation—A blood sample (10 mL) was collected from the jugular vein catheter immediately before (0 minutes) and at 15, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 minutes after each drug administration. Each blood sample was collected into a blood-collection tube and maintained at room temperature (approx 25°C) for 60 minutes. After centrifugation at 2,008 X g for 10 minutes, serum was transferred to sterile propylene tubes and stored at −20°C until analysis. Clinical evaluation (assessment of heart and respiratory rates and gastrointestinal tract motility) was performed at the same time points as blood sample collections and at 9, 12, and 24 hours after each drug administration.

Serum sample analysis—The concentrations of methadone in serum samples were determined by use of gas chromatography–mass spectrometry analysis as previously described.19 Briefly, aliquots of each serum sample were placed into separate tubes, and a method blank (water) and serum blanks with and without internal standard (dl-methadone-d5)20 were prepared. Methadone working standards (internal standard in metha-
nol) were prepared in concentrations of 0.1, 1, and 10 μg/mL, and calibration standards (working standard in drug-free equine serum) were prepared in concentrations of 2, 5, 10, 20, 50, 100, 250, and 500 ng/mL. The internal standard was added to aliquots of each control and test sample. After extraction and centrifugation, each sample was evaporated under a continuous stream of dry nitrogen and the resulting residue was dissolved in methylene chloride and transferred to a microinjection vial for gas chromatography–mass spectrometry analysis. Concentrations were determined by producing a calibration curve by use of the peak area ratios of the analyte (methadone; mass-to-charge ratio, 294) to the internal standard (methadone-d₃; mass-to-charge ratio, 297). Chromatographic data were processed by use of chromatography data system software. Limit of quantification was defined as the lowest concentration within approximately 20% of precision (limit of quantification, 2 ng/mL). Interassay and intra-assay coefficients of variation were 3.30% to 3.50% and 1.50% to 1.55%, respectively. The minimal acceptable correlation (R²) for standard curves was 0.998, and mean ± SD R² was 0.999 ± 0.001. Analyses were performed at the Analytical Systems Laboratory, School of Veterinary Medicine, Louisiana State University.

Pharmacokinetics analysis—A noncompartmental pharmacokinetics analysis was performed by use of pharmacokinetics software. Serum concentration data were generated for each horse, and the pharmacokinetic parameters, including terminal or elimination t₁/₂ₚ, AUC₀–∞, and MRT₀–∞, were determined by use of the trapezoidal model with linear interpolation. Systemic body clearance or Cl and Vd were corrected for F, for oral and intragastric routes of administration. Estimated Cₘₐₓ and Tₚₐₚ were determined directly from the estimated concentration-time curves obtained for different treatments. A linear regression analysis estimated the first-order rate constant associated with the terminal (log-linear) portion of the curve (λc) by use of as many as 13 log serum concentration-time points. Absolute F was calculated for each treatment (oral formulation administered orally or intragastrically and injectable formulation administered orally, IV, or intragastrically) as the ratio of total AUC from each formulation-route combination to the total AUC from the IV administration, and expressed as the following:

\[ F_{\text{Treatment}} = \frac{AUC_{\text{Treatment}}}{AUC_{\text{IV}}} \]

where AUCₜₖₙₐₜ is the AUC for each treatment (oral formulation administered orally or intragastrically and injectable formulation administered orally, IV, or intragastrically) and AUCₜₖₙₐₜ is the AUC for IV administration of the injectable formulation.

Results

Methadone hydrochloride was tolerated well by all horses after oral, intragastric, or IV administration. No behavioral changes or opioid-induced adverse effects, such as excitement, sedation, increased locomotor activity, and decrease of gastrointestinal tract motility, were observed during the 24-hour study period. Heart and respiratory rates were within reference limits during the entire study period in all horses.

After administration of each experimental treatment, methadone concentration was first measured in the sera of all horses at 13 minutes, and drug concentration was greater than the limit of quantification (2 ng/mL) for 6 hours after administration by all routes. Serum concentration-time curves generated after oral and intragastric administration of either formulation of methadone were characterized by a biphasic profile with rapid absorption and elimination phases describing a first-order process.
or among routes of administration. However, AUC\(0–t\), the estimated mean \(T_{\text{max}}\) for all administrations was significantly (<\(P\) 0.05) different. \(\text{P}_{\text{AUC}}\) and \(F\) were also significantly (<\(P\) 0.05) different.

\[\text{P}_{\text{AUC}} = \text{Body clearance corrected for } F, \quad \text{IG} = \text{Intragastric}, \quad V_{\text{F}} = \text{Apparent volume of distribution corrected for } F.\]

Data are reported as mean ± SD. For oral administrations, drug solution was directly squirted into the mouth by use of a syringe; for intragastric administrations, drug solution was delivered through a nasogastric tube. C/F = Clearance corrected for F, Vd/F = Apparent volume of distribution corrected for F. *Within a parameter, values with different superscript letters are significantly (<\(P\) 0.05) different.

**Assuming complete absorption (100%), the curve for IV administration revealed rapid distribution and elimination of the drug.** The area under the serum concentration-time curve for IV administration of the injectable formulation of methadone was used to estimate drug \(F\) for both oral and intragastric routes (Figure 1).

Mean ± SD estimated pharmacokinetic parameters after IV, oral, and intragastric administration were determined (Table 1). Estimated elimination \(\text{T}_{1/2}\), \(\text{MRT}_{\text{zero}}\), and \(\text{T}_{\text{max}}\) did not differ between methadone formulations or among routes of administration. However, AUC\(0–t\), Cl corrected for \(F\), V corrected for \(F\), C\(\text{max}\), and \(F\) were significantly (<\(P\) 0.05) different across treatments. The AUC and \(F\) were also significantly (<\(P\) 0.05) different between oral and injectable formulations after oral administration but not after intragastric administration. The estimated mean \(T_{\text{max}}\) for all administrations was between 65.5 ± 50.93 minutes and 105.0 ± 41.35 minutes. Methadone oral \(F\) was approximately 3 times as high as that of intragastric \(F\).

### Discussion

The single dose of 0.15 mg of methadone/kg was selected for use in horses in the present study on the basis of results of an investigation performed in our laboratory, which indicated that the pharmacokinetics of methadone are dose independent and that oral administration was not associated with opioid-induced adverse effects in horses. Effective or therapeutic concentrations of methadone in horses have not been reported, to our knowledge; however, plasma concentrations of methadone in the range of 33 to 59 ng/mL are efficacious in humans. As in the previous investigation, serum concentrations of methadone after oral and intragastric administration in horses in the present study were equivalent or higher to the effective concentration range reported for people.

In the present study, methadone was well tolerated by horses after administration of the drug via IV, oral, or intragastric routes. There were no adverse effects observed, including excitement, respiratory depression, increased locomotor activity, or decreased gastrointestinal tract motility.

As described for dogs and humans, individual variability with regard to the pharmacokinetics of methadone was also evident in horses. Methadone had a short elimination \(\text{T}_{1/2}\) of approximately 1 hour, short mean residence time, rapid Cl corrected for \(F\), and small \(C_{\text{max}}\) corrected for \(F\) in horses, indicating restricted distribution and rapid drug elimination. Changes in the Cl and \(V_{\text{d}}\) values between administration routes were more likely to be due to differences in \(F\) because these pharmacokinetic parameters were corrected for \(F\). The \(C_{\text{max}}\) and AUC but not \(T_{\text{max}}\) for methadone were significantly different between the oral and intragastric routes of administration. The rate was similar, but site of drug absorption was different. Studies in Beagles and Greyhounds have revealed similar patterns in parameters, including low oral \(F\) after single IV or oral administration of methadone. However, in contrast to findings of the present study, those dogs developed minimal adverse effects and plasma methadone concentrations were less than the therapeutic concentration (<40 ng/mL) 2 hours after drug administration.

To our knowledge, this is the first investigation of oral or intragastric administration of methadone in horses. We intended to determine drug \(F\) and possibly predict the absorption of methadone in horses. After oral (or intragastric) administration, highly lipophilic compounds like methadone are expected to be absorbed via passive transcellular transport mainly from the small intestine because of its large surface area and pH between 6 and 7.5. In addition to the physiologic properties of membranes, biochemical drug properties, including lipid solubility, degree of ionization, pKa, solution pH and formulation, and size and molecular weight of the compound, are determinant factors for drug absorption. Methadone has characteristics that contribute to high solubility and permeability, such as low molecular weight (<500 Da), no hydrogen-bond donors, single oxygen and nitrogen molecules, and octanol-water partition coefficient (log P) of approximately 5, which likely promote drug absorption. However, results of the present study suggested the absorption of methadone through the intestinal mucosa in horses was limited. Drug was placed directly into
the stomach of each study horse through a nasogastric tube, and it was immediately flushed with twice the volume of the tube capacity; however, low AUC and F of both oral and injectable formulations were detected after intragastric administration. These findings could be attributable to the first-pass metabolic effect, some drug adsorption to the tube, or another limiting factor for drug absorption. Genetic factors, including protein transporters, can play a major role in absorption and disposition of drugs, and the expression of P-glycoprotein in the apical membrane of enterocytes may be directly related to poor intestinal drug absorption. As an energy (ATP)-dependent efflux transmembrane protein transporter, P-glycoprotein is thought to limit absorption of several drugs by transporting them out of cells and back to the intestinal lumen. This protein is constitutively expressed in diverse tissues and species, and it may interfere with the disposition of several drugs, including opioids. However, little is known about this protein and its role in the absorption and disposition of methadone in horses.

Compared with the intragastric route, oral administration of methadone in the study horses resulted in higher plasma drug concentrations, AUC, and F; therefore, the oral cavity appeared to contribute considerably to the absorption of methadone through the oral mucosa. Methadone is a weak base with pKa of approximately 9.2, and the horses’ oral cavity (saliva) pH of 7 to 8 probably favored drug transport across the oral mucosa, compared with absorption from the stomach (pH, 1 to 2) or small intestine (interface pH, 5 to 6). Environmental pH and drug pKa determine the degree of ionization of the drug, and the higher the nonionized fraction of the drug, the greater its liposolubility, permeability, and absorption. For bases, the lower the pH of the environment below the pKa of the drug, the higher the ionized fraction. In addition, the high venous blood flow under the tongue was probably an important factor to favor a rapid and more complete absorption of methadone from the horses’ oral cavities.

Although the oral mucosa was the primary site of methadone absorption after oral administration because drug was delivered directly into horses’ mouth by use of a syringe, a prolonged drug serum concentration-time curve and oral F > 1.0 for the injectable formulation of methadone were some of the most interesting findings of the present study. This observation could have resulted from a superimposition of oral and intestinal absorption when part of the drug was swallowed faster, thereby allowing it shorter contact time with the oral mucosa or loss of some of the drug in the saliva.

Although methadone hydrochloride has characteristics that confer properties of high solubility and high permeability, absorption of the drug in the small intestine in horses appeared to be limited, probably due to the low drug F detected after intragastric administration. It is not known whether hepatic first-pass metabolism or the presence of the P-glycoprotein in the enterocytes of the small intestine is a limiting factor for intestinal absorption of methadone in horses. Nevertheless, administration of methadone could benefit pain management in horses, and further studies should be performed to better understand drug absorption and the analgesic properties of methadone in this species.

References


Methadone hydrochloride has a molecular weight of 345.19 g/mol, and both formulations used in the present study differed in their composition with regard to the inactive ingredients only, which apparently do not interfere with the disposition of the drug. However, via oral administration, the injectable formulation (pH, 2.28) of methadone seemed to be better absorbed than the oral formulation (pH, 2.62), possibly because of a higher dissociation of nonionized fraction favored by the higher delivery solution’s pH. In addition, horses salivated more with the administration of the oral formulation by mouth, which could explain the different serum concentrations and Fs between formulations. We believe the flavoring component of the inactive ingredients imparts a strong smell and taste to the oral formulation, which was not well accepted by the horses. Possibly, it could have caused the oral formulation to be swallowed faster, thereby allowing it shorter contact time with the oral mucosa or loss of some of the drug in the saliva.

Methadone hydrochloride has characteristics that confer properties of high solubility and high permeability, absorption of the drug in the small intestine in horses appeared to be limited, probably due to the low drug F detected after intragastric administration. It is not known whether hepatic first-pass metabolism or the presence of the P-glycoprotein in the enterocytes of the small intestine is a limiting factor for intestinal absorption of methadone in horses. Nevertheless, administration of methadone could benefit pain management in horses, and further studies should be performed to better understand drug absorption and the analgesic properties of methadone in this species.

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