### Title

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# Preliminary study of the pharmacokinetics, tissue distribution, and behavioral and select physiological effects of morphine 6-glucuronide (M6G) following intravenous administration to horses

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## Abstract

Although morphine has demonstrated antinociceptive effects in horses, its administration has been associated with dose-dependent adverse effects. In humans and rats, part of the analgesic effect of morphine has been attributed to the active metabolite, morphine-6-glucuronide (M6G). Although morphine can cause several undesirable effects, M6G has a more favorable safety profile. The objective of this study was to characterize the pharmacokinetics, tissue distribution, and behavioral and select physiological effects of M6G following intravenous administration to a small group of horses. In Part 1 of the study, 3 horses received a single intravenous administration of saline, 0.5 mg/kg body weight (BW) M6G, or 0.5 mg/kg BW morphine in a 3-way crossover design. Blood samples were collected up to 96 hours post-administration, concentrations of drug and metabolites measured, and pharmacokinetics determined. Behavioral and physiological effects were then recorded. In Part 2 of the study, 2 horses scheduled to be euthanized for other reasons, were administered 0.5 mg/kg BW M6G. Blood, cerebrospinal fluid (CSF), and various tissue samples were collected post-administration and concentrations of drug were determined. The clearance of M6G was more rapid and the volume of distribution at steady state was smaller for M6G compared to morphine. A reaction characterized by head shaking, pawing, and slight ataxia was observed immediately following administration of both morphine and M6G to horses. After M6G administration, these behaviors subsided rapidly and were followed by a longer period of sedation. Following administration, M6G was detected in the kidney, liver, CSF, and regions of the brain. Results of this study encourage further investigation of M6G in order to assess its clinical feasibility as an analgesic in horses.

## *Résumé*

*Bien que la morphine ait démontré des effets antinociceptifs chez les chevaux, son administration a été associée avec des effets non-désirés d'une manière dose-dépendante. Chez les humains et les rats, une partie de l'effet analgésique de la morphine a été attribuée au métabolite actif, morphine-6-glucuronide (M6G). Bien que la morphine puisse causer plusieurs effets indésirables, M6G a un profil de sécurité plus favorable. L'objectif de cette étude était de caractériser la pharmacocinétique, la distribution tissulaire, et le comportement et sélectionner des effets physiologiques de M6G suivant son administration intraveineuse à un petit groupe de chevaux. Dans la Partie 1 de l'étude, trois chevaux ont reçu l'administration intraveineuse d'une dose unique de saline, 0,5 mg/kg de poids corporel (BW) de M6G, ou 0,5 mg/kg BW de morphine selon un essai croisé à trois voies. Des échantillons sanguins ont été prélevés jusqu'à 96 h post-administration, les concentrations de drogues et de métabolites mesurées, et les pharmacocinétiques déterminées. Les effets physiologiques et sur le comportement ont par la suite été notés. Dans la Partie 2 de l'étude, deux chevaux devant être euthanasiés pour d'autres raisons, ont reçu 0,5 mg/kg BW de M6G. Du sang, du liquide céphalo-rachidien (CSF), et différents échantillons de tissu ont été prélevés post-administration et les concentration de drogue furent déterminées. La clairance de M6G a été plus rapide et le volume de distribution à l'état d'équilibre était plus petit pour M6G comparativement à la morphine. Une réaction caractérisée par le tremblement de la tête, du piaffage, et une légère ataxie a été observée immédiatement à la suite de l'administration soit de morphine ou de M6G aux chevaux. Après administration de M6G, ces comportements diminuèrent rapidement et furent suivis par une période plus longue de sédation. À la suite de l'administration, M6G a été détecté dans les reins, le foie, le CSF, et des régions du cerveau. Les résultats de cette étude incitent à réaliser des études additionnelles sur M6G afin d'évaluer son potentiel clinique comme analgésique chez les chevaux.*

*(Traduit par Docteur Serge Messier)*

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# Introduction

Pain management for equine patients is limited. Although opioids are a frequent and well-characterized drug class used for analgesia in other species, their unpredictable and often undesirable side effects limit their use in horses (1). The pharmacodynamics of morphine have been described in horses following intravenous and intramuscular administration (2–4). Although it has demonstrated antinociceptive effects (5), intravenous administration of morphine has been associated with dose-dependent excitatory effects on the central nervous system (CNS) (3,4,6) and unwanted gastrointestinal effects occurring at what is believed to be a therapeutic dose of approximately 0.2 mg/kg body weight (BW) (5).

In humans, morphine undergoes extensive glucuronidation to morphine 3-glucuronide (M3G; 60%) and morphine 6-glucuronide (M6G; 6% to 10%) (7). In both humans and rats, at least part of the analgesic effects of morphine has been attributed to the M6G metabolite (8). Studies in humans and rats have shown that M6G has a greater affinity for the *mu* receptor compared to morphine (8,9) and, following intrathecal administration, the analgesic potency of M6G is reportedly 100-fold higher than morphine (10,11). Interestingly, although M6G is highly polar, it appears able to cross the blood-brain barrier (BBB), as evidenced by studies in rats describing concentrations of the metabolite in brain tissue (12).

Whereas morphine can cause a number of unfavorable effects in humans (respiratory depression, nausea, and vomiting), M6G has a more favorable safety profile (7,13). In contrast to M6G, M3G is believed to be devoid of analgesic properties and does not appear to bind to opioid receptors (14). Furthermore, it has been postulated that M3G antagonizes the analgesic effects of morphine and may have neuroexcitatory effects (15–18).

The metabolism and pharmacokinetics of morphine in the horse have been studied previously (3,4,19). As reported in humans and rats, horses metabolize morphine to M3G and M6G and produce higher concentrations of M3G than humans (3,4). If M6G contributes to the analgesic effects of morphine in horses and M3G causes excitation, administration of M6G may prove to be an effective analgesic that is devoid of the excitatory effects observed following morphine administration in horses.

Based on this and previous studies in other species, we hypothesized that M6G would be able to enter the central nervous system following administration of the metabolite to horses. To that end, the objective of this study was to characterize the pharmacokinetics and tissue distribution of M6G and its behavioral and select physiological effects in a small group of horses following intravenous administration.

# Materials and methods

## Part 1: Pharmacokinetics and behavioral and physiological effects

***Animals.*** Three healthy university-owned thoroughbred geldings (aged 3 to 8 y) weighing 510 kg ± 43 (average ± SD) were used for this pilot study. Horses did not receive any medications for a minimum of 2 wk before the study. A complete blood (cell) count, serum biochemistry, and physical examination were conducted to confirm the health of the horses. The Institutional Animal Care and Use Committee of the University of California, Davis approved this study (#22516).

***Instrumentation and drug administration.*** This study was conducted in a randomized, 3-way balanced crossover design with a minimum 2-week washout between treatments. In each phase, horses were randomly assigned to 1 of 3 groups and were given a single intravenous dose of 0.5 mg/kg BW morphine sulfate, 0.5 mg/kg BW M6G, or 5 mL BW (comparable volume to M6G) of saline.

Since the pharmacokinetics of M6G have not been previously reported in the horse, an M6G dose of 0.5 mg/kg BW was selected based on the dose of morphine administered in a previous study conducted by our laboratory in which an excitatory behavioral response was observed (3). Morphine 6-glucuronide powder (Toronto Research Chemical, North York, Ontario) was purchased and subsequently compounded for intravenous administration. The powder was weighed, dissolved in sterile Lactated Ringer's Solution at a concentration of 50 mg/mL, and filter-sterilized in a sterile hood. The solution was administered within 20 min of mixing.

Each horse was weighed prior to drug administration. Due to the potential for ileus associated with opioid administration in horses, animals were fasted for 12 h prior to administration of the drug and for 4 h post-administration. Water was available *ad libitum.* A 14-gauge catheter was placed in each jugular vein using the aseptic technique prior to drug administration. One catheter was used for drug administration, whereas the other was used for sample collection.

***Sample collection.*** Blood (10 mL) was collected at times 0, 5, 10, 15, 30, 45 min, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 18, 24, 36, 48, and 96 h post-administration into EDTA blood tubes (Kendall/Tyco Healthcare, Mansfield, Massachusetts, USA) and placed on ice until centrifugation (maximum of 1 h). Catheters were removed after collection of the 24-hour sample, with the remaining samples collected *via* direct venipuncture. Samples were centrifuged at 3000 × *g* at 4°C for 10 min, plasma immediately transferred to cryovials (Phoenix Research Products, Chandler, North Carolina, USA), and samples stored at −20°C. An aliquot of each blood sample was taken at times 0, 5, 10, 15, 30, 45 min, 1, 2, 4, 6, and 8 h post-administration of drug for determination of packed cell volume (PCV) *via* microhematocrit and total protein (TP) *via* refractometer. Each packed cell volume and total protein measurement was taken in duplicate with the average recorded for each time point.

***Drug concentration determination.*** Concentrations of morphine and metabolites were measured using a previously validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (3,4).

***Pharmacokinetic calculations.*** Pharmacokinetic parameters for morphine and M6G were determined using non-compartmental analysis and commercially available software (Phoenix WinNonlin Version 8.0; Certara, Princeton, New Jersey, USA). Non-compartmental analysis was used, as previous studies have demonstrated non-linear elimination of morphine at higher doses (3). The area under curve (AUC) from time 0 to infinity ($AUC_{0\rightarrow\infty}$) was obtained by using the linear up log down trapezoidal rule, then dividing the last plasma concentration by the terminal slope extrapolated to infinity.

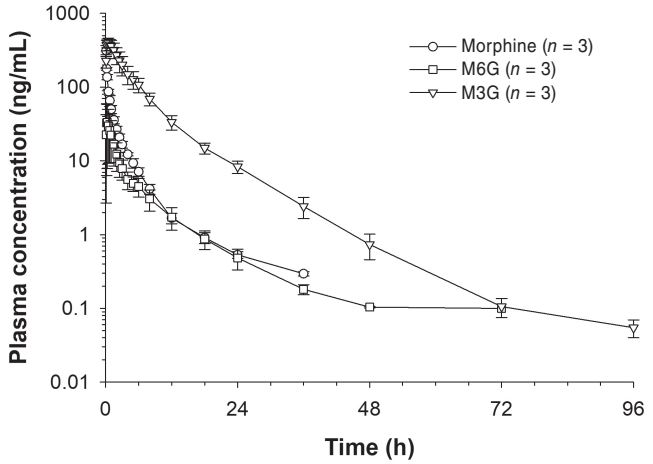


**Figure 1. Plasma concentration time curve for morphine, morphine 6-glucuronide (M6G), and morphine 3-glucuronide (M3G) following intravenous administration of 0.5 mg/kg BW morphine to 3 horses.**

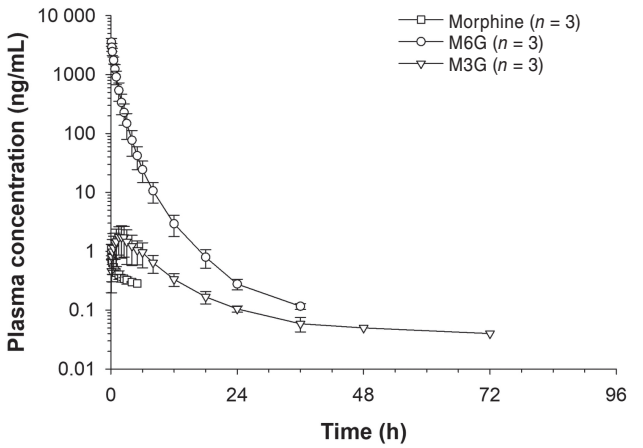


**Figure 2. Plasma concentration time curve for morphine, morphine 6-glucuronide (M6G), and morphine 3-glucuronide (M3G) following intravenous administration of 0.5 mg/kg BW M6G to 3 horses.**

***Behavioral and physiological responses.*** Notable post-administration physiological and behavioral responses were noted and recorded continuously for the first 2 h and then hourly for the next 4 h. After the initial 6 h of each study day, direct observations were noted at minimum in the morning and evenings (same time each day) for the next 4 d. Step counters and Holter monitors were used for 6 h following treatment to assess excitatory behavior, as described in a previous study (3).

To evaluate gastrointestinal (GI) behavior, each abdominal quadrant was assigned a GI borborygmi score ranging from 0 to 4, with 0 being absent and 4 being increased sounds. The GI scores were assessed prior to and at 30 and 45 min, and 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h post-administration. Defecation frequency and fecal consistency were also recorded throughout the 6-hour sampling period.

***Statistical analysis.*** Commercially available software (Stata/IC 17.0; StataCorp LP, College Station, Texas, USA) was used to determine significant differences in pharmacodynamic parameters. Differences between baseline and each time point and between each treatment group at each time point were evaluated using a mixed-effects analysis of variance, with the horse as the random effect and time and treatment as the fixed effects. *Post-hoc* comparisons were accomplished with a Bonferroni multiple comparison adjustment to preserve a nominal significance level of 0.05.

## Part 2: Distribution of M6G in tissue

Two horses that were to be euthanized, 1 for neurologic and 1 for orthopedic reasons, were administered a single IV dose of 0.5 mg/kg BW of M6G, formulated as described for Part 1. Blood samples were collected prior to drug administration and at 5, 10, 15, 30, 45 min, and 1 h post-administration. One hour post-administration, the horses were euthanized with pentobarbital.

Blood, cerebral spinal fluid, and tissues, including kidney (right for horse #1 and right and left for horse #2), liver, cerebral cortex, thalamus, caudal brainstem, cerebellum, and trigeminal ganglia, were collected and stored at −20°C until processed. Blood samples were processed as described previously for the blood samples in Part 1. The Institutional Animal Care and Use Committee of the University of California, Davis approved this study (#22110).

**Table I. Pharmacokinetic parameters (mean ± SD) for morphine following a single IV administration of 0.5 mg/kg BW to adult horses. All values reported were generated using non-compartmental analysis.**

| | 0.5 mg/kg BW morphine IV | | |
|---|---|---|---|
| Parameters | Horse 1 | Horse 2 | Horse 3 |
| C(0) ng/mL | 525.7 | 376.1 | 809.2 |
| $Lambda_z$ (1/h) | 0.11 | 0.06 | 0.06 |
| HL $Lambda_z$ (h) | 6.50 | 11.9 | 12.9 |
| VDss (L/kg BW) | 6.13 | 7.30 | 6.98 |
| CL (mL/min/kg BW) | 30.4 | 29.8 | 30.9 |
| $AUC_{0-inf}$ (h*ng/mL) | 274.3 | 279.3 | 269.9 |

C(0) — Concentration extrapolated to the origin; $Lambda_z$ — Terminal slope; HL $Lambda_z$ — Terminal half-life; VDss — Volume of distribution at steady-state; CL — Total systemic clearance; $AUC_{0-inf}$ — Area under the plasma-concentration curve from time 0 to infinity.

***Tissue drug concentration determination.*** Approximately 100 mg of tissue (90 to 140 mg) was weighed into tared Precellys hard tissue homogenizing vials (Omni International, Kennesaw, Georgia, USA) and 1 mL of the internal standard (d3-morphine-6BD glucuronide) was added. The samples were homogenized twice at 4.5 m/s for 30 s in an Omni Bead Ruptor Elite tissue homogenizer (Omni International), transferred to microcentrifuge tubes, and centrifuged at 14 000 rpm (12 753 g) for 5 min. The supernatant (500 µL) was dried under nitrogen and reconstituted in 150 µL of 5% acetonitrile in water with 0.2% formic acid, centrifuged again as before, and 20 µL injected into the liquid chromatography-mass spectrometry (LC-MS/MS) system. The concentrations of morphine, M3G, and M6G were measured by LC-MS/MS, as described in previous studies (3,4).

**Table II. Pharmacokinetic parameters (mean ± SD) for morphine-6 glucuronide (M6G) following a single IV administration of 0.5 mg/kg BW morphine-6 glucuronide or 0.5 mg/kg BW morphine to adult horses. All values reported were generated using non-compartmental analysis.**

| | Horse 1 | | Horse 2 | | Horse 3 | |
|---|---|---|---|---|---|---|
| Treatment | M6G | Morphine | M6G | Morphine | M6G | Morphine |
| C(0) ng/mL | 3675 | NA | 4998 | NA | 4416 | NA |
| $Lambda_z$ (1/h) | 0.205 | 0.100 | 0.202 | 0.078 | 0.080 | 0.087 |
| HL $Lambda_z$ (h) | 3.39 | 6.96 | 3.42 | 8.99 | 8.62 | 8.01 |
| VDss (L/kg BW) | 2.24 | NA | 1.61 | NA | 1.94 | NA |
| CL (mL/min/kg BW) | 2.83 | NA | 2.18 | NA | 3.28 | NA |
| $AUC_{0-inf}$ (h*ng/mL) | 2925 | 86.1 | 3817 | 80.3 | 2530 | 167.4 |

C(0) — Concentration extrapolated to the origin; $Lambda_z$ — Terminal slope; HL $Lambda_z$ — Terminal half-life; VDss — Volume of distribution at steady-state; CL — Total systemic clearance; $AUC_{0-inf}$ — Area under the plasma-concentration curve from time 0 to infinity.
NA — Not available.

# Results

The precision and accuracy of the assay were determined by assaying quality control samples in replicates ($n = 6$) for each analyte. Accuracy and precision were within 10% of the expected value and considered acceptable based on the U.S. Food and Drug Administration's guidelines for Bioanalytical Method Validation (20). The technique was optimized to provide a limit of quantitation (LOQ) of 0.25 ng/mL and 1 ng/mL and a limit of detection of approximately 0.1 ng/mL and 0.5 ng/mL in blood and tissues/cerebrospinal fluid.

Plasma concentrations of morphine and metabolites following morphine administration are depicted in Figure 1 and concentrations following M6G administration in Figure 2. Plasma pharmacokinetic parameters for morphine following intravenous administration of 0.5 mg/kg BW are shown in Table I and parameters for M6G following administration of 0.5 mg/kg BW morphine or 0.5 mg/kg BW M6G are shown in Table II. The clearance of M6G was more rapid than morphine and the volume of distribution at steady state was smaller for M6G compared to morphine. The terminal half-life for M6G following administration of morphine was longer than the terminal half-life following M6G administration.

A reaction characterized by head shaking, pawing, and slight ataxia was observed within the first 5 min of M6G administration, followed by a longer period of sedation. Following morphine administration, horses also exhibited head shaking and pawing, which was followed by a longer period of these behaviors compared to the M6G group. The number of steps taken per 10 minutes following saline, morphine, and M6G administration is depicted in Figure 3 A, B, and C, respectively. Following administration of morphine, the number of steps recorded increased for about 120 min (Figure 3 B). Following M6G administration, the number of steps initially increased (first 10 min), relative to baseline, although not significantly, and then subsequently decreased (Figure 3 C).

Heart rate was significantly ($P < 0.05$) increased relative to baseline from 5 min to 3 h and then again at 5 and 6 h following M6G administration and from 5 min until 6 h post-administration of morphine (Table III). Packed cell volume and total protein were significantly increased, relative to baseline, at several times post-administration in all 3 treatment groups (Table IV).

Gastrointestinal sounds were significantly reduced ($P < 0.05$), relative to baseline, from 30 min until 2 h, then again at 3 h following morphine administration (Table V). Following M6G administration, gastrointestinal sounds were decreased from 5 min to 2 h and again at 3 h post-administration (Table V). Fecal output was decreased relative to baseline in the morphine dose group in 2 out of 3 horses for up to 8 h post-administration. Fecal output remained consistent, compared to baseline in the saline and M6G treatment groups.

In Part 2 of the study, following a single IV administration of 0.5 mg/kg BW, M6G was detected in the kidney, liver, CSF, and various regions of the brain (Table VI). The highest concentrations in brain tissue were found in the trigeminal ganglia in both horses (Table VI).

# Discussion

As in previous studies in horses, in which M3G was the predominant metabolite following morphine administration, concentrations of M3G far exceeded M6G concentrations in the present study. Following M6G administration, low concentrations of both morphine and M3G were noted. This observation has been reported before in humans (21), with investigators theorizing that production of M3G and morphine following M6G administration may be a result of enterohepatic recirculation. Although further study would be necessary to definitively conclude this in horses, this is a possible explanation for the identification of the 2 compounds following M6G administration in the present study.

The volume of distribution of M6G (1.61 to 2.24 L/kg) was markedly smaller compared to morphine (6.13 to 7.30 L/kg), as would be expected based on the polarity and larger molecular weight of M6G. The systemic clearance of morphine was rapid compared to M6G, which is likely due to a rapid rate of biotransformation of morphine to both M6G and M3G, as has been described for other species (22). For 2 out of 3 horses studied, the terminal half-life of M6G following intravenous administration of morphine was longer compared to intravenous administration of M6G. This difference may

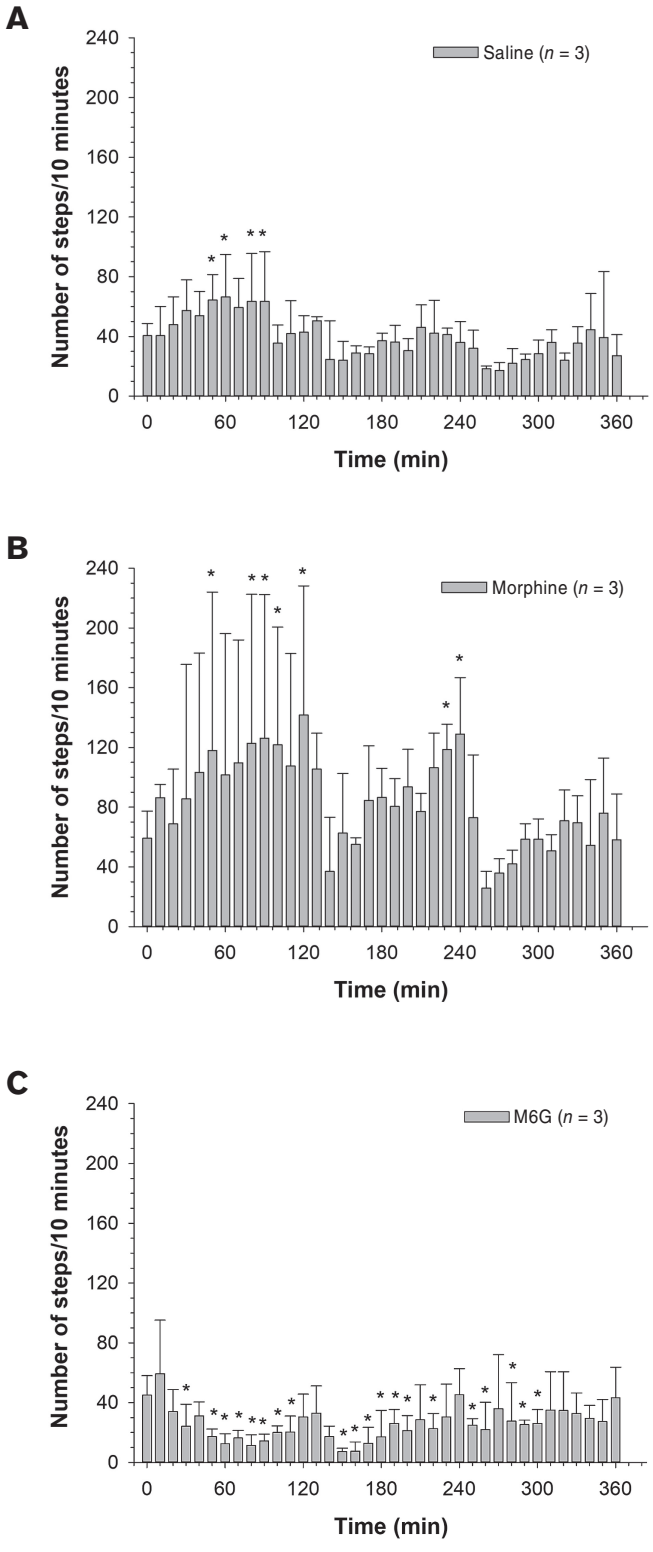


**Figure 3. Number of steps taken per 10 minutes shown in bars (mean ± SD) following a single intravenous administration of (A) saline, (B) 0.5 mg/kg BW morphine, or (C) 0.5 mg/kg BW morphine 6-glucuronide (M6G) to 3 adult horses.**

* **Indicates a significant difference ($P < 0.05$) relative to baseline.**

**Table III. Heart rate (beats/min; mean ± SD) following a single IV administration of saline, 0.5 mg/kg BW morphine-6 glucuronide (M6G), or 0.5 mg/kg BW morphine to 3 adult horses.**

| Time (h) | Saline | 0.5 mg/kg BW M6G | 0.5 mg/kg BW morphine |
|---|---|---|---|
| 0 | 28.3 ± 4.6 | 29.3 ± 3.8 | 27.0 ± 3.0 |
| 0.03 | 29.3 ± 4.7[b,c] | 43.7 ± 7.6*[,a] | 42.0 ± 4.6*[,a] |
| 0.08 | 28.3 ± 5.7[b,c] | 44.7 ± 8.0*[,a] | 43.7 ± 5.0*[,a] |
| 0.13 | 30.0 ± 3.5[b,c] | 48.7 ± 0.6*[,a] | 53.0 ± 12.8*[,a] |
| 0.17 | 27.7 ± 6.5[b,c] | 47.0 ± 5.0*[,a] | 47.0 ± 8.7*[,a] |
| 0.20 | 31.0 ± 4.6[c] | 45.7 ± 5.9* | 48.0 ± 14.7*[,a] |
| 0.25 | 28.3 ± 8.0[b,c] | 44.3 ± 5.5*[,a] | 44.3 ± 5.0*[,a] |
| 0.33 | 32.0 ± 3.6[b,c] | 43.0 ± 3.6*[,a] | 42.0 ± 8.7*[,a] |
| 0.5 | 31.0 ± 7.5*[,b,c] | 42.0 ± 1.0*[,a] | 44.0 ± 7.5*[,a] |
| 0.75 | 28.7 ± 8.0[c] | 38.7 ± 1.5* | 49.3 ± 11.9*[,a] |
| 1 | 30.0 ± 5.6[b,c] | 38.3 ± 2.5*[,a] | 40.7 ± 2.1*[,a] |
| 1.25 | 31.7 ± 7.0[c] | 39.0 ± 3.6* | 44.0 ± 2.0*[,a] |
| 1.5 | 33.7 ± 3.0*[,b,c] | 39.0 ± 2.0*[,a,c] | 42.7 ± 2.9*[,a,b] |
| 2 | 30.0 ± 8.2[b,c] | 36.7 ± 3.5*[,a,c] | 42.7 ± 2.0*[,a,b] |
| 2.5 | 34.7 ± 4.9*[,b,c] | 41.3 ± 3.2*[,a] | 42.3 ± 3.0*[,a] |
| 3 | 33.3 ± 3.2*[,b,c] | 37.7 ± 5.5*[,a,c] | 41.7 ± 4.7*[,a,b] |
| 4 | 34.0 ± 5.0*[,c] | 33.0 ± 10.5[c] | 40.3 ± 8.5*[,a,b] |
| 5 | 42.3 ± 3.8* | 37.3 ± 4.5* | 40.3 ± 8.0* |
| 6 | 36.3 ± 2.5* | 41.3 ± 2.0* | 41.3 ± 8.5* |

* Indicates a significant difference ($P < 0.05$) relative to baseline.
[a] Indicates a significant difference ($P < 0.05$) from saline group.
[b] Indicates a significant difference ($P < 0.05$) from M6G group.
[c] Indicates a significant difference ($P < 0.05$) from morphine group.

be attributable to the time it takes the body to metabolize morphine to M6G. This rate of conversion of morphine to M6G may be slower than the elimination of M6G, resulting in a flip-flop effect.

In the third horse, the elimination half-life of M6G following morphine administration and following M6G administration was in close agreement. Although the reason for the discrepancy between this horse and the other 2 horses is not clear, in humans, similar to what was calculated for the third horse, the elimination half-life of M6G following morphine administration and following direct administration of M6G were not different (23).

It should also be noted that the number of horses studied was small and additional study, with a larger sample size, would be necessary to draw any definitive conclusions regarding a flip-flop effect. Although the terminal half-life of M6G, whether following morphine or direct M6G administration, is longer than that reported in humans (23), the half-life of M6G following morphine administration is in agreement with previous studies in horses (3,4).

Morphine administration to horses has been associated with a dose-dependent excitatory effect (3,4). As reported previously in this study, intravenous administration of 0.5 mg/kg BW of morphine resulted in a prolonged (120 min post-administration) increase in locomotion and heart rate. Although M6G administration resulted in a brief period (10 min) of signs consistent with excitation, this response was transient and was followed by behavior consistent with sedation. Heart rate also increased following administration

**Table IV. Packed cell volume (PCV) and total protein concentration (TP) (mean ± SD) at specified time points following administration of a single dose of saline (5 mL), 0.5 mg/kg morphine BW, or 0.5 mg/kg BW morphine 6-glucuronide (M6G) to 3 horses.**

| | Saline | | 0.5 mg/kg BW M6G | | 0.5 mg/kg BW morphine | |
|---|---|---|---|---|---|---|
| Time | PCV | TP | PCV | TP | PCV | TP |
| 0 min | 36.5 ± 0.8 | 5.9 ± 0.2 | 34.5 ± 4.6 | 6.2 ± 0.7 | 35.9 ± 2.2 | 6.1 ± 0.5 |
| 5 min | 34.6 ± 1.0*,b,c | 6.0 ± 0.2* | 41.3 ± 3.3*,a | 6.3 ± 0.8 | 41.4 ± 1.6*,a | 6.3 ± 0.6* |
| 15 min | 35.0 ± 3.4b,c | 6.1 ± 0.0* | 40.7 ± 4.0*,a | 6.5 ± 0.7 | 41.8 ± 1.3*,a | 6.3 ± 0.5* |
| 30 min | 34.1 ± 3.8*,b,c | 6.1 ± 0.0* | 37.5 ± 3.5*,a | 6.4 ± 0.7 | 38.9 ± 1.7*,a | 6.4 ± 0.5* |
| 45 min | 33.8 ± 3.7*,c | 6.1 ± 0.2* | 36.4 ± 2.8 | 6.4 ± 0.7 | 39.7 ± 1.0*,a | 6.3 ± 0.5* |
| 1 h | 32.8 ± 2.9*,c | 6.0 ± 0.2* | 34.7 ± 4.4 | 6.4 ± 0.7 | 37.0 ± 2.2a | 6.4 ± 0.6* |
| 2 h | 32.5 ± 2.6*,c | 6.1 ± 0.2* | 32.2 ± 4.0c | 6.3 ± 0.6 | 36.6 ± 2.5a,b | 6.3 ± 0.3* |
| 4 h | 35.7 ± 2.3c | 6.3 ± 0.2* | 36.8 ± 4.5c | 6.5 ± 0.9* | 40.1 ± 2.8*,a,b | 6.5 ± 0.4* |
| 6 h | 39.0 ± 2.5*,c | 6.3 ± 0.0*,b,c | 41.0 ± 2.0* | 6.7 ± 0.5*,a | 42.5 ± 2.0*,a | 6.9 ± 0.5*,a |
| 8 h | 36.6 ± 1.4b,c | 6.0 ± 0.2*,b | 43.1 ± 2.8a | 6.7 ± 0.6*,a | 42.2 ± 1.8*,a | 6.5 ± 0.5* |

* Indicates a significant difference ($P < 0.05$) relative to baseline.

[a] Indicates a significant difference ($P < 0.05$) from saline group.

[b] Indicates a significant difference ($P < 0.05$) from M6G group.

[c] Indicates a significant difference ($P < 0.05$) from morphine group.

**Table V. Gastrointestinal scores (mean ± SD) following a single IV administration of saline, 0.5 mg/kg BW morphine-6 glucuronide (M6G), or 0.5 mg/kg BW morphine to 3 horses.**

| Time (h) | Saline | 0.5 mg/kg BW M6G | 0.5 mg/kg BW morphine |
|---|---|---|---|
| 0 | 0.7 ± 0.7 | 1.0 ± 0.0 | 1.0 ± 0.0 |
| 0.25 | 0.7 ± 0.7b,c | 0.0 ± 0.0*,a | 0.0 ± 0.0*,a |
| 0.5† | 1.0 ± 0.0 | 0.0 ± 0.0* | 0.0 ± 0.0* |
| 0.75 | 0.7 ± 0.7b,c | 0.0 ± 0.0*,a | 0.0 ± 0.0*,a |
| 1.0 | 0.7 ± 0.7 | 0.3 ± 0.7* | 0.0 ± 0.0* |
| 1.5 | 0.7 ± 0.7b,c | 0.0 ± 0.0*,a | 0.0 ± 0.0*,a |
| 2.0† | 1.0 ± 0.0 | 0.0 ± 0.0* | 0.0 ± 0.0* |
| 2.5 | 1.3 ± 0.7 | 0.7 ± 0.7 | 0.7 ± 0.0 |
| 3.0 | 1.7 ± 0.0b,c | 0.3 ± 0.7*,a | 0.3 ± 0.7*,a |
| 4.0 | 1.3 ± 0.0 | 1.3 ± 0.0 | 0.7 ± 0.0 |
| 5.0 | 1.3 ± 0.0 | 1.3 ± 0.0 | 1.0 ± 0.7 |
| 6.0 | 1.3 ± 0.0 | 1.3 ± 0.7 | 0.7 ± 0.7 |

† Statistical model could not fit data.

* Indicates a significant difference ($P < 0.05$) relative to baseline.

[a] Indicates a significant difference ($P < 0.05$) from saline group.

[b] Indicates a significant difference ($P < 0.05$) from M6G group.

[c] Indicates a significant difference ($P < 0.05$) from morphine group.

of M6G, but less so compared to the increase observed following morphine administration. These results suggest that M6G has less of a central excitatory effect than morphine.

Both packed cell volume (PCV) and total protein increased from baseline following administration of morphine and M6G. This finding is in agreement with our previous study in horses, in which PCV and total protein increased following administration of an intravenous dose of 0.5 mg/kg BW morphine (3). In this previous study, although the authors acknowledged that environmental factors, specifically warm summer temperatures, could have led to mild dehydration and subsequent changes in PCV and total protein, they also add further support to the theory that this finding is a result of increased sympathetic tone and splenic contraction (3). In extreme circumstances, this response has been shown to nearly double PCV in horses (24,25).

It is important to note that environmental temperatures were also high in the present study and dehydration cannot be excluded as a potential explanation for the increases in both PCV and total protein noted. The increase in heart rate observed in both the M6G and morphine dose groups in this study, however, further supports the theory that these effects are related to drug administration. If changes in PCV and total protein (TP) were due to dehydration, it would be expected that they would continue to increase with time and that there would be both quantitative and qualitative changes in urinary output, but this was not, at least casually, noted.

The effects of opioids such as morphine on the gastrointestinal (GI) system have been well-described in horses. In agreement with previous studies, administration of morphine did appear to decrease GI motility (3,6,26). Interestingly, the same effect was seen in the present study following administration of M6G. Although adverse effects on the GI tract are reportedly less following administration of M6G in humans (27), this finding is not completely unexpected in the present study given the affinity of M6G for opioid receptors and the knowledge that binding to opioid receptors is thought to alter motility, secretion, absorption, and blood flow in the GI tract. It is important to note that fasting the horses in the present study may have also contributed in some small measure to the decrease in GI motility. This was not seen in the saline-dose group, which suggests that this effect is related to the administration of morphine and M6G.

As the reported analgesic effects of M6G suggest that it can cross the blood brain barrier, in Part 2 of this study, we sought to describe the tissue distribution of M6G following IV administration. Although highly polar, previous studies in rats describe M6G's ability to cross the blood-brain barrier (BBB) in animals (12,28,29).

**Table VI. Blood (A) and tissue (B) concentrations of morphine 6-glucuruonide (M6G), morphine 3-glucuronide (M3G), and morphine following intravenous administration of 0.5 mg/kg BW morphine 6-glucuronide (M6G) to 2 horses.**

**A)**

| | Concentration (ng/mL) | | | | | |
|---|---|---|---|---|---|---|
| | M6G | | M3G | | Morphine | |
| Time (min) | Horse 1 | Horse 2 | Horse 1 | Horse 2 | Horse 1 | Horse 2 |
| 0 | ND | ND | ND | ND | ND | ND |
| 5 | 3479.4 | 2835.0 | 0.66 | 2.01 | 0.81 | 5.83 |
| 10 | 2907.1 | 2369.7 | 0.80 | 2.47 | 0.56 | 5.24 |
| 15 | 3022.3 | 2009.1 | 1.04 | 2.63 | 0.71 | 4.57 |
| 30 | 1803.3 | 1449.3 | 1.32 | 2.81 | 0.39 | 3.78 |
| 45 | 1277.1 | 1080.2 | 1.58 | 3.01 | 0.39 | 2.75 |
| 60 | 953.4 | 870.4 | 1.93 | 3.29 | 0.38 | 2.42 |

**B)**

| | Concentration (ng/g) | | | | | |
|---|---|---|---|---|---|---|
| | M6G | | M3G | | Morphine | |
| Tissue | Horse 1 | Horse 2 | Horse 1 | Horse 2 | Horse 1 | Horse 2 |
| Kidney | 3313.8 | 2488.4 (right)<br>2683.0 (left) | 39.1 | 37.0 (right)<br>24.4 (left) | 15.5 | 36.6 (right)<br>28.3 (left) |
| Liver | 332.8 | 400.5 | 2.23 | 3.88 | ND | < LOQ |
| Cerebral cortex (occipital lobe) | 33.3 | 11.3 | ND | ND | ND | ND |
| Cerebral cortex (temporal lobe) | 49.3 | 12.8 | ND | ND | ND | ND |
| Thalamus | 32.6 | 11.7 | ND | ND | ND | ND |
| Caudal brainstem | 23.5 | 10.2 | ND | ND | ND | ND |
| Cerebral cortex (front) | 36.7 | 26.2 | ND | ND | ND | ND |
| Cerebellum | 38.6 | 25.7 | ND | ND | ND | ND |
| Trigeminal ganglia | 348.1 | 280.8 (right)<br>304.8 (left) | ND | ND | ND | < LOQ (left)<br>< LOQ (right) |
| Cerebrospinal fluid | 10.8 | 2.95 | ND | ND | ND | ND |

LOQ — Limit of quantitation.
ND — Not detected.

Morphine-6-glucuronide has been reported in brain tissue and cerebral spinal fluid following subcutaneous administration of 10 mg/kg BW of M6G to rats (12). Similarly, in the present study, M6G was detected in homogenates from the occipital, temporal, and frontal lobe and the thalamus, cerebellum, and brainstem following IV administration, which suggests that M6G can cross the blood-brain barrier in horses.

Since its hydrophilic nature would presumably prevent diffusion, it has been suggested that the ability of M6G to cross the BBB is the result of transport proteins, such as Oatp2 and GLUT-1 (30,31). An additional hypothesis is that the drug molecule may be able to fold and mask its polar groups, thereby increasing its lipophilicity and allowing it to cross the BBB and enter the central nervous system (32). Although M6G appears to be a substrate for some ATP-binding cassette (ABC) transporter multidrug resistance proteins, namely MRP2 and MRP3 (efflux proteins), in *in-vivo* studies, it does not appear to be a substrate for P-glycoprotein, the efflux protein present within the BBB (33–35).

Appreciable concentrations of M6G were also exhibited in the trigeminal ganglia in both horses in Part 2 of this study. Notably, entry into the trigeminal ganglia is easier as there is no BBB that must be crossed. The clinical implications of this are not clear. Although there is a large concentration of *mu* opioid receptors in the trigeminal ganglia, studies in humans describing the effectiveness of opioids such as morphine in treating pain conditions associated with the trigeminal ganglia have been inconclusive (36). This has not yet been reported in horses. It is not surprising that high concentrations of M6G were found in the kidneys, since they are the primary organ of elimination of both M6G and M3G in other species (37,38).

It is important to note the limitations of the present study, i.e., the number of horses studied was small and only a single dose was assessed. The results of this study provide preliminary information and are supportive of further investigation of M6G. Additional studies are necessary with more horses, varying doses, and an assessment of the effects of this compound on nociception in order to further assess its clinical feasibility as an analgesic in horses.

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