Received: 29 November 2023 | Revised: 26 April 2024 | Accepted: 8 June 2024

DOI: 10.1111/vsu.14128

WILEY

CLINICAL RESEARCH

Evaluation of pharmacokinetics of metoclopramide administered via subcutaneous bolus and intravenous constant rate infusion to adult horses

Amy M. Brandon DVM[1] | **Jarred M. Williams DVM, MS, PhD, DACVS (Large Animal), DACVECC (Large Animal)**[1] | **Jen L. Davis DVM, PhD, DACVIM (Large Animal), DACVCP**[2] | **Emily G. Martin DVM, PhD, DACVS (Large Animal)**[3] | **Ava M. Capper DVM**[1] | **Naomi E. Crabtree DVM, MS, DACVS (Large Animal), DACVECC (Large Animal)**[1]

[1]Department of Large Animal Medicine, University of Georgia, Athens, Georgia, USA

[2]Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, Virginia, USA

[3]Department of Clinical Sciences, North Carolina State University, Raleigh, North Carolina, USA

Correspondence
Amy M. Brandon, Department of Large Animal Medicine, 2200 College Station Road, Athens, GA 30602, USA.
Email: amy.brandon.dvm@gmail.com

Funding information
University of Georgia

Abstract

Objective: To determine the pharmacokinetics (PK) of metoclopramide administered via intravenous continuous rate infusion (IV CRI) and subcutaneous (SC) bolus and evaluate for gastrointestinal motility and adverse side effects.

Study design: Experimental study; randomized, crossover design.

Animals: Six healthy adult horses.

Methods: Each horse received metoclopramide via IV CRI (0.04 mg/kg/h for 24 h) and SC bolus (0.08 mg/kg once), with ≥1 week washout period between. Plasma was analyzed by UPLC-MS/MS. Compartmental modeling was used to determine PK parameters for each treatment; nonparametric superposition was used to simulate multiple SC bolus regimens. Gastrointestinal motility and evidence of adverse effects were monitored.

Results: T_{max} (h) for SC bolus was 0.583 ± 0.204 versus 17.3 ± 6.41 for IV CRI, while C_{max} (ng/mL) was 27.7 ± 6.38 versus 43.6 ± 9.97, respectively. AUC (h × ng/mL) was calculated as 902 ± 189 for 24 h IV CRI versus 244 ± 37.4 simulated for 0.08 mg/kg SC bolus every 8 h. Simulations revealed similar exposure between groups with administration of 0.96 mg/kg/day SC bolus, divided into three, four, or six doses. SC bolus bioavailability was estimated as $110 \pm 11.5\%$. No clear trends in motility alteration were identified. No adverse effects were noted.

The results of this report were presented as an abstract at the International Veterinary Emergency and Critical Care Symposium on September 9, 2023.

Conclusion: Repeated SC boluses of metoclopramide at 0.08 mg/kg would result in lower total drug exposure and T_{max} than IV CRI administration but would be highly bioavailable.

Clinical significance: Higher and/or more frequent SC bolus doses are needed to achieve a similar AUC to IV CRI. No adverse effects were noted; however, evaluation of alternative dosing strategies is warranted.

1 | INTRODUCTION

Gastrointestinal (GI) disorders are some of the most common ailments addressed by equine clinicians, often resulting in disruptions to the normal intestinal motility patterns, such as ileus.[1,2] Historically, the term "postoperative ileus" (POI) has been used to describe any perceived reduction in intestinal function that results in postoperative reflux, although this term is likely an oversimplification. Historically, POI occurs in 10%–47% of surgical colic cases, with the mortality rate of horses with POI as high as 87%.[2–9] Another study recorded a seven-fold increase in euthanasia in horses with POI when compared to horses without ileus.[1]

Metoclopramide is a prokinetic drug used to combat ileus.[1–4,7,9–16] It has been shown to improve jejunal motility and gastric emptying in the normal horse, as well as reduce nasogastric reflux in clinically affected horses.[1,3,4,7,10,12,14–16] Metoclopramide is a benzamide drug which exerts its effects via a number of mechanisms. It acts as an antagonist at dopamine receptors, a mixed agonist–antagonist at serotonin receptors (5-HT4 agonist, 5-HT3 antagonist), exerts a mild blockade of alpha-2 receptors, and has limited direct stimulation of the smooth muscle within the gastrointestinal tract via action on acetylcholine receptors.[1,3,4,7,11,12,14] Additionally, because metoclopramide crosses the blood–brain barrier, the effect of dopamine receptor antagonism is not restricted to the gastrointestinal tract and can result in undesirable extrapyramidal side effects such as restlessness, excitation, and irritability. These effects can progress to dangerous, aggressive behavior that may preclude the continued use of metoclopramide in the horse.[1–4,9,12,13,16]

Many doses and administration routes have been reported for metoclopramide in equine patients[1–4,7,10–12,15,16]; however, pharmacokinetic data is lacking and doses are extrapolated from other species.[4,12,16] The most commonly used administration protocol for metoclopramide in horses, an intravenous continuous rate infusion (IV CRI) at 0.04 mg/kg/h, comes from a retrospective study published in 1996.[4] This dosing regimen yielded clinical improvement in nasogastric reflux and had fewer serious side effects than previous studies investigating higher doses.[2–4,11]

Other dosing regimens have been anecdotally used in horses, with some practitioners administering metoclopramide as a repeated subcutaneous (SC) bolus in an effort to decrease extrapyramidal effects. At these authors' institutions, a dose of 0.08 mg/kg every 8 h SC bolus has been used, despite no documented evidence that this dose is therapeutically appropriate or minimizes side effects.

The objectives of this study were to define the pharmacokinetic properties of the traditional IV CRI dosing and a novel SC bolus dosing regimen of metoclopramide in healthy horses, to evaluate for the effects on GI motility, and to document the incidence of extrapyramidal effects.

2 | MATERIALS AND METHODS

2.1 | Study subjects

Six healthy, university-owned, adult horses (four geldings, two mares) of various breeds (four Quarter Horses, one Appendix, one Thoroughbred) were used following Institutional Animal Care and Use Committee (IACUC) review and approval of the protocol. Horses weighed between 415.9–561.4 kg (median 488.7 kg), and ages ranged from 8 to 20 years (median 16 years). Prior to inclusion in the study, all horses were confirmed to have no history of respiratory or gastrointestinal disease, and a physical examination was performed by a licensed veterinarian to ensure no evidence of current gastrointestinal or other severe systemic disease. There was also no history of any drug administration within the week prior to the study. Horses were transported to the hospital for the duration of each treatment period. Upon arrival, each horse was weighed to ensure accurate dosing. Following this, horses were housed for one night in the hospital to acclimate them to the environment prior to initiation of monitoring and treatment protocols. During the treatment and sample collection periods, horses were housed in box stalls. The horses were fed one flake of grass hay every 6 h throughout the study period, which was of the same type, from the same supplier, as they were fed prior to inclusion in the study, with continuous free access to fresh, clean water. The diet and feeding schedule remained unchanged

throughout the horses' time in the hospital, regardless of monitoring or treatment protocol. The study was performed as a crossover design, with a washout period of at least 1 week between treatments. Horses were randomly assigned to treatment regimens using an online randomization program such that two horses received the IV CRI treatment protocol first, followed by the SC bolus treatment protocol, while four horses received the SC bolus treatment protocol first, followed by the IV CRI treatment protocol (https://www.randomizer.org/).

2.2 | Drug administration, sample collection, and analysis

2.2.1 | IV CRI treatment protocol

For the IV CRI regimen, a small area over both jugular veins was clipped and aseptically prepared with chlorhexidine scrub and alcohol. A 14-gauge, 5.25-inch intravenous catheter (MILA, Florence, Kentucky) was placed in each jugular vein using aseptic technique and sutured in place (2–0 Supramid Extra II, S. Jackson, Inc., Alexandria, Virginia) to facilitate drug administration (right jugular vein) and sample collection (left jugular vein). The metoclopramide dose (0.04 mg/kg/h[5]) was calculated based on the horse's weight and 24-h worth of metoclopramide was then added to 1 L of sterile saline (0.9% NaCl) that had previously had the same volume removed. During administration to the first two horses, each pump completed infusion early. The pumps were assessed, and it was determined that the early completion was because some of the volume intended for administration was used to prime the fluid pump tubing and extension sets rather than due to error in pump volume administration. All future IV CRI bags were made to contain 25 h worth of metoclopramide added to 1 L of sterile saline (0.9% NaCl) as above. The delivery rate was calculated to ensure appropriate administration of 0.04 mg/kg/h via the fluid pump. Following this change in methodology, all remaining IV CRIs ran for the full 24-h period as planned.

For blood sample collection, 10 mL of blood was aspirated from the catheter in the left jugular vein and discarded. Following collection of a 20 mL blood sample, the catheter was flushed with 10 mL of sterile, heparinized saline. A blood sample was obtained just prior to the initiation of treatment, and then additional blood samples at 15, 30, and 60 min, and 2, 4, 6, 8, 12, 18, and 24 h throughout the IV CRI. Following discontinuation of the IV CRI, samples were again obtained at the same time points, as well as at 36 and 48 h post-discontinuation, to evaluate clearance and elimination of the drug. The intravenous catheters were removed following the 24-h sample, and the 36- and 48-h samples were obtained via direct venipuncture.

2.2.2 | SC bolus treatment protocol

For the SC bolus administration regimen, a catheter was placed into the left jugular vein as described above to facilitate sample collection. The SC bolus metoclopramide dose (0.08 mg/kg) was calculated based on the horse's weight. The horse was restrained with a halter and lead rope, and the metoclopramide was administered subcutaneously in the left cervical region using a 22 gauge 1 inch needle. All horses received the subcutaneous injection from the same licensed veterinarian to standardize injection location and technique.

Blood samples were collected, as described above, just prior to injection, and then at 15, 30, and 60 min, and 2, 4, 6, 8, 12, 18, 24, 36, and 48 h post-injection. The intravenous catheters were removed following the 24-h sample, and the 36- and 48-h samples were obtained via direct venipuncture.

2.2.3 | Sample handling and analysis

Blood samples were collected in EDTA plasma tubes and kept on ice for no more than an hour prior to processing. The samples were centrifuged at 2500 rpm (699 × *g*) for 10 min; the plasma was harvested and stored in polypropylene cryotubes at −80°C until analysis. Concentrations of metoclopramide were determined by ultra-high pressure liquid chromatography with tandem mass spectrometry (UPLC-MS/MS), using a method adapted from Lee et al.[17] and validated in the same laboratory using the procedure described by Shah et al.[18] Calibration curves were made daily using blank equine plasma fortified with stock drug solution over a plasma concentration range of 0.1–200 ng/mL for metoclopramide. The coefficient of determination (R^2) for each curve was >0.99 and all concentrations were within ±15% of the actual concentration. The system had a limit of detection (LOD) of approximately 0.02 ng/mL, as determined by a signal-to-noise ratio of three using blank plasma. The limit of quantification (LOQ) was set at the lowest concentration on the individual calibration curves (0.1 ng/mL) Quality control samples were run using five replicates at 0.5, 10 and 200 ng/mL. At those concentrations, interday accuracy was 96.6%, 98.5% and 99.9%, respectively. Interday precision was 1.49%, 2.76% and 2.01%, respectively.

Drug concentrations were analyzed using commercially available software (Phoenix WinNonlin version 6.2, Certara USA, Inc., Princeton, New Jersey) to determine

pharmacokinetic parameters (including C_{max}, AUC, and half-life) for each horse and for each route of administration. The model was chosen based on the best overall fit for the data from all horses for each route using visual inspection of the semi-logarithmic plasma concentration versus time curves, as well as Akaike Information Criterion (AIC) and Schwarz Bayesian Criterion (SBC) goodness of fit measurements. Additional information on goodness of fit can be found in Figures S1 and S2. The final model chosen for the IV CRI data was a two-compartment model with IV infusion input, first-order elimination, micro-constants and no lag time (Phoenix model 9) and weighting at $1/\hat{y}$.[10] The final model chosen for SC bolus administration was a two-compartment open model with first-order input and elimination, microconstants and lag time (Phoenix model 12) and weighting at $1/y$.[10] The results of two-compartment models are reported in the manuscript. The intravenous CRI data also fit well with a three-compartment model (see Table S1); however, the data from the two-compartment model was used in the manuscript to allow direct comparison with the SC bolus data. The following equation was used for calculation of bioavailability: $F(\%) = [(AUC_{SC0\text{-}24hsim} \times D_{IV})/(AUC_{CRI0\text{-}24h} \times D_{SC})] \times 100$, where $AUC_{SC0\text{-}24hsim}$ is the AUC simulated from single dose data using the study dose ($D_{SC} = 0.08$ mg/kg) administered every 8 h for three doses; the $AUC_{CRI0\text{-}24h}$ is the AUC measured during the 24 h of drug infusion using the study dose ($D_{IV} = 0.04$ mg/kg/h). Simulations of plasma concentrations following repeated SC bolus administrations at various doses and frequencies, each designed to administer the same total 24-h metoclopramide dose as the IV CRI, were performed with the same pharmacokinetic software utilizing nonparametric superposition. This method assumed each drug dose acts independently, and that linear pharmacokinetics apply with multiple doses, such that the rate and extent of absorption, as well as total drug clearance do not change with changes in dose or dosing interval. From the simulated plasma concentrations, an accumulation ratio was computed for the different doses and intervals input into the program using the slope of the terminal elimination phase (λ_z) from noncompartmental modeling. Noncompartmental modeling was also used to determine maximum, minimum, and average plasma concentrations, as well as an $AUC_{0\text{-}24}$ for the simulated dosing regimens, and this data was compared to a 24-h IV CRI.

2.3 | Motility and adverse effects monitoring

The day following admission to the hospital and prior to initiation of either treatment protocol, each horse was monitored for a 24-h period to establish normal behavioral and gastrointestinal motility parameters prior to drug administration. Throughout the course of the IV CRI, as well as for 24 h following drug administration via either route, close monitoring of behavioral and gastrointestinal motility parameters was performed.

2.3.1 | Gastrointestinal motility

Ultrasonography was used to provide a semi-quantitative measure of small intestinal motility as described in previous studies.[5,8,10,19–22] Initially, four ultrasonographic windows were identified and clipped to facilitate repeated examination of the same sites: left inguinal region, right inguinal region, the left 14th, 15th intercostal spaces (ICS) at the level of the spleen and stomach, and the right dorsal to mid-15th and 16th ICS at the level of the right kidney and duodenum. Ultrasonographic examination of all four windows was performed on each of the six horses while undergoing their first treatment protocols. At this midway point, the data from the ultrasonographic examinations were reviewed prior to each of the horses undergoing the next treatment protocol. It was noted that both inguinal windows and the window on the left at the level of the spleen and stomach inconsistently identified small intestinal loops. Sometimes, no small intestine would be visualized at all within the window for the duration of the ultrasonographic examination, and at other times, multiple loops would be visualized simultaneously, clouding the ability to reliably use the window to objectively, repeatedly assess and compare motility. Thus, for the remaining treatment protocols, only the duodenal window was assessed, and this was the only ultrasonographic window included in data analysis. A portable, handheld ultrasound (GE VScan Air, GE HealthCare, Chicago, Illinois) was used by a member of the study team to evaluate the window every 2 h; all ultrasonographic examinations were reviewed by the primary author. During each examination, the duodenum was positively identified and then observed for 2 min. The number of duodenal peristaltic contractions, defined as the lumen fully opening followed by fully closing, was recorded.

Gastrointestinal auscultation was performed every 2 h by a member of the study team. Auscultation was performed as described in other studies[21–23]; briefly, each of the four abdominal quadrants were auscultated for 30 s, and the number of short (<3 s) borborygmi during that time period was counted. The number of borborygmi from each quadrant was recorded individually but was also summed at each time point to provide the total number of borborygmi for each time point.

Frequency of defecation was monitored via review of video recordings (see below).

2.3.2 | Behavioral side effects

Over the course of the day prior to drug administration, the 24-h duration of the IV CRI, and the 24 h post drug administration (in either group), horses were continuously recorded in addition to hourly monitoring via direct visualization. Video recordings were reviewed after conclusion of the hospitalization period by a single observer. The following behavioral effects were noted: pacing a full circle around the stall, relocating within the stall (<1 full circle of movement), flehmen response, kicking at abdomen, pawing, flank watching, bowing, and laying down. If laying down was noted, the number of times the horse was noted to be sternally versus laterally recumbent was recorded. The frequency of defecation and urination identified in the footage was also recorded. The cumulative number of occurrences of each clinical sign for each 24-h period (prior to drug administration, during intravenous infusion, following drug administration) was recorded following review of all records and camera footage.

2.3.3 | Statistical analysis

All analyses were performed by a statistician using SAS 9.4 (Cary, North Carolina). A significance threshold of 0.05 was used. Generalized linear mixed models (GLMM) were used to analyze borborygmi, duodenal contractions, and side effect counts. A negative binomial link function was used as appropriate for count data. The models for borborygmi and duodenal contractions included fixed factors of route, period, and time and all two- and one three-way interaction effects. The model for each side effect included fixed factors of route, period, and a route by period interaction effect. All models had a random intercept for each horse. Tests of route differences post-administration (for each time if applicable) and of period at each route (and time if applicable) were tested. Comparisons for each endpoint were adjusted for multiple comparisons using the linear step-up method of Benjamini and Hochberg (1995). Satterthwaite degrees of freedom method and residual pseudo-likelihood estimation were used.

3 | RESULTS

3.1 | Pharmacokinetic analysis

Data were best fitted to a two-compartment open model as described above. The mean plasma concentrations of metoclopramide over time following IV CRI and SC bolus administration are shown in Figure 1. The T_{max} (h) for SC bolus was 0.583 ± 0.204 compared to 17.3 ± 6.41 for IV CRI. The C_{max} (ng/mL) for the SC bolus dose was 27.7 ± 6.38, as compared to 43.6 ± 9.97 for the IV CRI. The AUC (area under the curve; h × ng/mL) of the 24-h IV CRI (902 ± 189) was compared to that simulated for 0.08 mg/kg SC bolus administered every 8 h for three doses (244 ± 37.4). Based on comparison of the AUC over the course of the CRI time frame (0.96 mg/kg over a 24 h period) and the dose corrected AUC simulated for 0.08 mg/kg SC bolus administered every 8 h (0.24 mg/kg over a 24 h period), bioavailability (%) for the subcutaneous route of administration was high (110 ± 11.5). Additional PK data are listed in Table 1.

The simulated SC bolus dosing regimens (0.16 mg/kg every 4 h, 0.24 mg/kg every 6 h, and 0.32 mg/kg every 8 h) were designed to administer the same total 24 h metoclopramide concentration as the IV CRI (0.96 mg/kg over 24 h). The simulations assumed linear pharmacokinetics over the doses. The data from the simulations is available in Table 2, and the simulated mean plasma concentrations of metoclopramide over time are represented in Figure 2. The maximum and average concentrations of metoclopramide for each simulated SC bolus dosage protocol are represented in Figure 3. Simulations revealed similar exposure (as represented by the calculated AUC) between the IV CRI group (902 ± 189 h × ng/mL) and the simulations of 0.16 mg/kg every 4 h (935 h × ng/mL), 0.24 mg/kg every 6 h (946 h × ng/mL), and 0.32 mg/kg every 8 h (978 h × ng/mL). All simulated doses were bioequivalent to both each other and the IV CRI group.

3.2 | Gastrointestinal motility

The mean number of contractions recorded (Figure 4) during ultrasonographic examination was significantly higher at 2 h post SC bolus injection compared to the same time of day during the 24 h of monitoring prior to drug administration (11.3 and 5.7 respectively, $p = .006$). No other significant differences were noted between treatment groups or the 24 h of monitoring prior to drug administration.

The mean number of borborygmi (Figure 5) were significantly higher at 16 and 22 h post SC bolus than they were at the same time points following discontinuation of the IV CRI (16 h: 31.5 for SC bolus vs. 22 for IV CRI, $p = .048$; 22 h: 30.7 for SC bolus vs. 18.8 for IV CRI, $p = .011$). The SC bolus group also had a significantly higher number of mean borborygmi at 2- and 4-h post-injection compared to auscultations performed at the same times of day during the 24 h of monitoring prior to

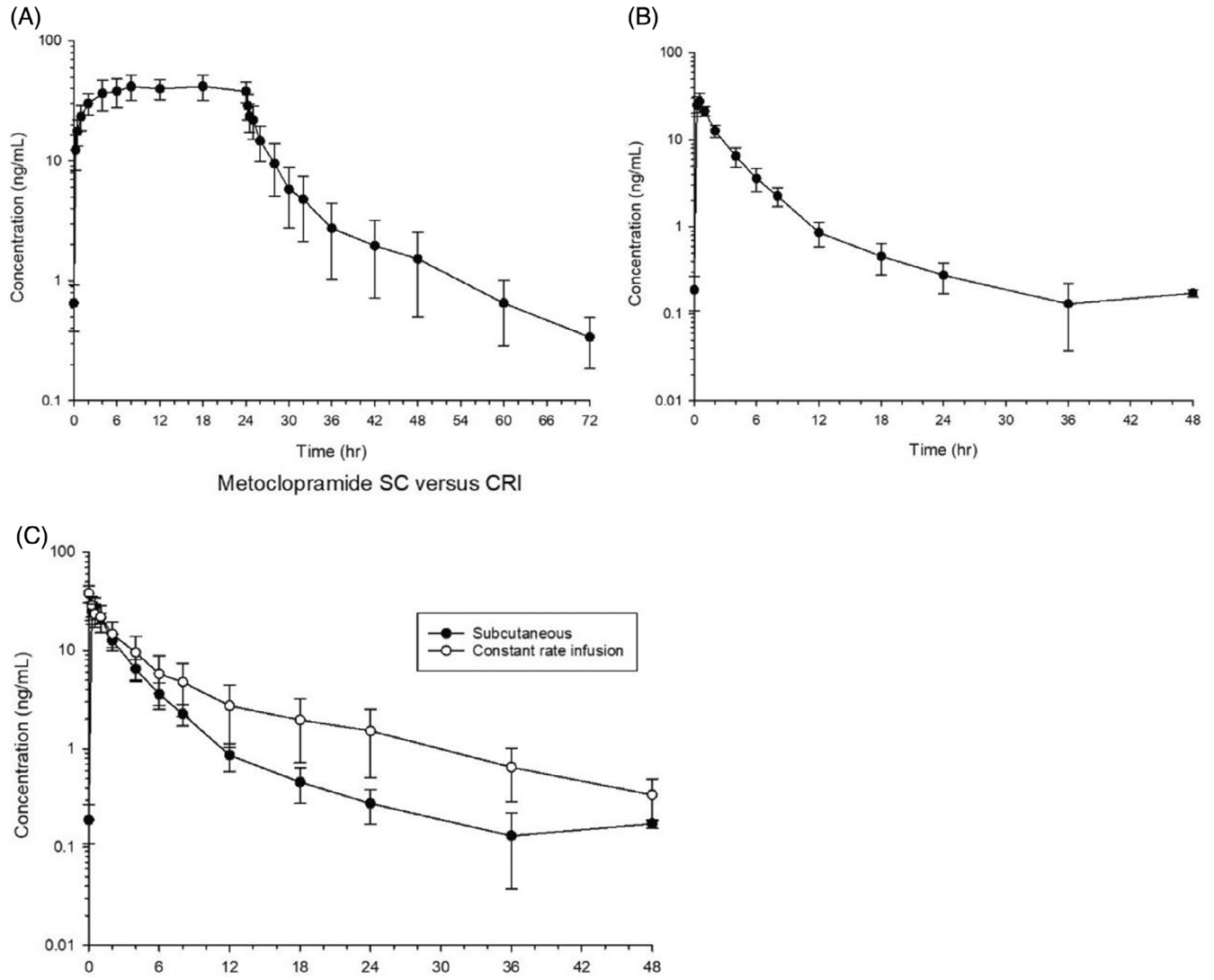

FIGURE 1 (A) Concentration of metoclopramide over time during and following a 24-h intravenous continuous rate infusion (IV CRI) at 0.04 mg/kg/h. The CRI was initiated at time 0. At the 24 h point, drug administration was discontinued. (B) Concentration of metoclopramide over time following a single, subcutaneous (SC) bolus of 0.08 mg/kg. The drug was administered at time 0. (C) Concentrations of metoclopramide over time following discontinuation of the IV CRI (open circle) and administration of the single SC bolus (solid circle).

drug administration (2 h post-injection: 30.2 on the day of drug administration vs. 18.2 the previous day, $p = .006$; 4 h post-injection: 28.8 on the day of drug administration vs. 18.7 the previous day, $p = .030$). The mean number of borborygmi during IV CRI was significantly higher at 16 h of infusion compared to auscultation at the same time of day during the 24 h of monitoring prior to drug administration or post-infusion (32 during IV CRI vs. 26 the day before or 22 the day after IV-CRI, $p = .048$). The times borborygmi were noted to be statistically significant in the treatment groups were not correlated with the blood concentrations of metoclopramide. They did correspond with times the horses were fed, although not all feeding times carried a statistically significant increase in mean number of borborygmi. No other significant differences in mean borborygmi were noted between treatment groups or the 24 h of monitoring prior to drug administration.

No significant differences in fecal production were noted at any time point between treatment groups (during IV CRI: median of 15 and range of 13–23 defecations per 24 h; post-IV CRI median: median of 15.5 and range of 10–20 defecations per 24 h; post-SC bolus injection: median of 12.5 and range of 10–16 defecations per 24 h) or the 24 h of monitoring prior to drug administration (median 14.5; range 10–24 defecations per 24 h).

3.3 | Behavioral side effects

No adverse behavioral effects were noted in either treatment groups and observed behaviors did not differ

TABLE 1 Two-compartmental pharmacokinetic parameters for metoclopramide administration to six horses, either via IV CRI at 0.04 mg/kg/h for 24 h or a single 0.08 mg/kg SC bolus.

Pharmacokinetic variable	Constant rate infusion (mean ± SD)	Subcutaneous (mean ± SD)
T_{lag} (h)	–	0.071 ± 0.090
C_{max} (ng/mL)[a]	43.6 ± 9.97	27.7 ± 6.38
T_{max} (h)[a]	17.3 ± 6.41	0.583 ± 0.204
A (ng/mL)	991 ± 240	30.4 ± 3.86
α (h^{-1})	1.13 ± 0.13	0.478 ± 0.140
B (ng/mL)	19.2 ± 12.1	2.03 ± 0.903
β (h^{-1})	0.07 ± 0.004	0.083 ± 0.021
$t_{1/2\alpha}$ (h)	0.62 ± 0.068	1.55 ± 0.423
$t_{1/2\beta}$ (h)	9.83 ± 0.616	9.08 ± 3.58
k_{10} (h^{-1})	0.873 ± 0.036	0.361 ± 0.081
k_{12} (h^{-1})	0.235 ± 0.137	0.089 ± 0.057
k_{21} (h^{-1})	0.092 ± 0.015	0.111 ± 0.035
k_{10} $t_{1/2}$ (h)	0.795 ± 0.033	1.99 ± 0.395
$AUC_{0-\infty}$ (h × ng/mL)	1160 ± 281	86.6 ± 13.8
Cl (mL/kg/min)	14.5 ± 3.52	–
Vd_{ss} (L/kg)	3.51 ± 1.72	–
AUC_{0-24} (h × ng/mL)	902 ± 189	244 ± 37.4[b]
F%	–	110 ± 11.5[b]
AIC	0.109 ± 12.9	−36.1 ± 15.3
SBC	4.34 ± 13.1	−33.6 ± 15.4

Abbreviations: A, coefficient of the distribution phase; AIC, Aikaike information criterion; $AUC_{0-\infty}$, area under the concentration-time curve extrapolated to infinity; B, coefficient of the elimination phase; Cl, total clearance; C_{max}, maximum plasma concentration; F%, bioavailability (based on AUC_{0-24}); k_{10} $t_{1/2}$, half-life of elimination; k_{10}, k_{12}, k_{21}, microdistribution rate constants; IV CRI, intravenous continuous rate infusion; SBC, Schwarz's Bayesian criterion; SC, subcutaneous; $t_{1/2\alpha}$, half-life of distribution; $t_{1/2\beta}$, half-life of elimination; T_{lag}, lag time; T_{max}, time to maximum plasma concentration; Vd_{ss}, volume of distribution at steady state; α, distribution phase rate constant; β, elimination phase rate constant.
[a]Taken directly from the data.
[b]Simulated data using nonparametric superposition for three doses of 0.08 mg/kg SC every 8 h.

significantly from the 24 h of monitoring prior to drug administration.

4 | DISCUSSION

The pharmacokinetics of the 0.08 mg/kg SC bolus dose differed from those of the IV CRI at 0.04 mg/kg/h, suggesting that this particular SC bolus dosing strategy would not provide an equivalent clinical effect to the IV CRI. However, since metoclopramide was rapidly absorbed following SC bolus administration and the estimated bioavailability was high, SC bolus administration may be a viable option for drug delivery if appropriate dosing strategies can be identified. The simulated SC bolus protocols (0.16 mg/kg SC bolus every 4 h, 0.24 mg/kg SC bolus every 6 h, and 0.32 mg/kg SC bolus every 8 h) did achieve the same total drug delivery as the IV CRI over a 24-h period based on similarities in the AUC, although each exhibited differences in other parameters such as total drug dose or accumulation index. Although no adverse effects were noted in either treatment group in the present study, the safety of these simulated regimens requires further study.

Metoclopramide given at 0.08 mg/kg as a SC bolus had a substantially lower C_{max} (27.7 ± 6.38 ng/mL) and drug exposure, as represented by the AUC for simulated dosing every 8 h (244 h × ng/mL), compared to the IV CRI (43.6 ± 9.97 ng/mL and 902 ± 189 h × ng/mL, respectively). It is therefore likely that this dosing strategy would not provide clinically comparable effects to the IV CRI. An important consideration when interpreting these data is that, in the absence of a known therapeutic target concentration for metoclopramide, the assumption has been made that the concentration achieved by the IV CRI at 0.04 mg/kg/h is required to produce a clinically significant prokinetic effect. While an objective motility assessment of the IV CRI dosing strategy has not been performed to date, it has been reported to decrease nasogastric reflux, and thus presumably improve gastric and/or small intestinal function in clinical cases.[4] As such, this was considered the most appropriate point of comparison for the present study. Various studies have assessed higher IV CRI rates, but to the authors' knowledge, no studies have assessed clinical efficacy of lower rates.[1,4,11,12,16] Therefore, it is possible that a lower CRI dose may be efficacious in clinical cases, in which case the conclusions drawn here regarding the likely efficacy of the tested and simulated SC bolus protocols may not be entirely accurate. An additional consideration in interpretation of this study is that steady-state concentrations, which occur after 4–5 half-lives, were not reached during the 24-h IV CRI administration. Although plasma concentrations did appear to plateau prior to discontinuation of the CRI, these results cannot be interpreted as equivalent to achieving steady-state concentrations. This study showed rapid absorption (as indicated by T_{max}) of SC metoclopramide and a high calculated bioavailability, making SC metoclopramide an intriguing avenue for further investigation. However, this bioavailability may not accurately reflect the absolute bioavailability of SC metoclopramide, as bioavailability is typically calculated based on an IV bolus dose, but no IV bolus was administered to the horses in this study due to concerns regarding

TABLE 2 Simulated pharmacokinetic parameters for multiple SC dosing regimens of metoclopramide.

	0.08 mg/kg every 8 h[a]	0.16 mg/kg every 4 h[a]	0.24 mg/kg every 6 h[a]	0.32 mg/kg every 8 h[a]	IV CRI 0.04 mg/kg/h[b]
C_{max} (ng/mL)	27.4	54.4	79.3	109	43.6 ± 9.97
C_{min} (ng/mL)	2.26	12.9	10.8	9.04	
C_{avg} (ng/mL)	9.28	29.4	33.8	37.3	39.8 ± 8.48
$AUC_{0\text{-}24}$ (h × ng/mL)	244	935	946	978	902 ± 189
AI	1.19	1.45	1.21	1.19	

Note: See key for Table 1 for remaining parameter definitions.
Abbreviations: AI, accumulation index; C_{avg}, average plasma concentration over dosing interval; C_{min}, minimum plasma concentration; IV CRI, intravenous continuous rate infusion; SC, subcutaneous.
[a]Simulated data.
[b]Taken directly from the data.

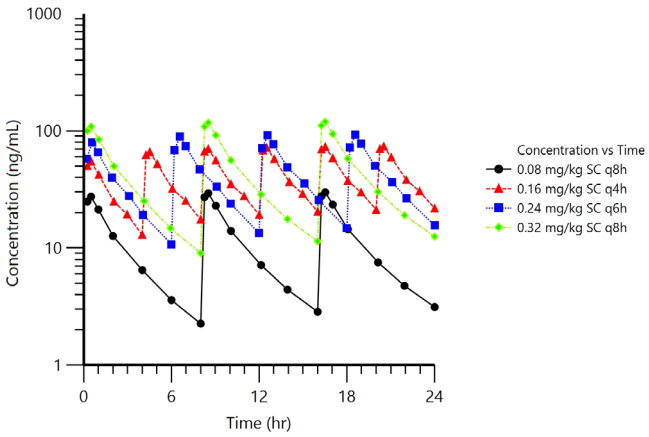

FIGURE 2 Concentrations of metoclopramide over time for simulated, repeated subcutaneous (SC) bolus dosing regimens of metoclopramide.

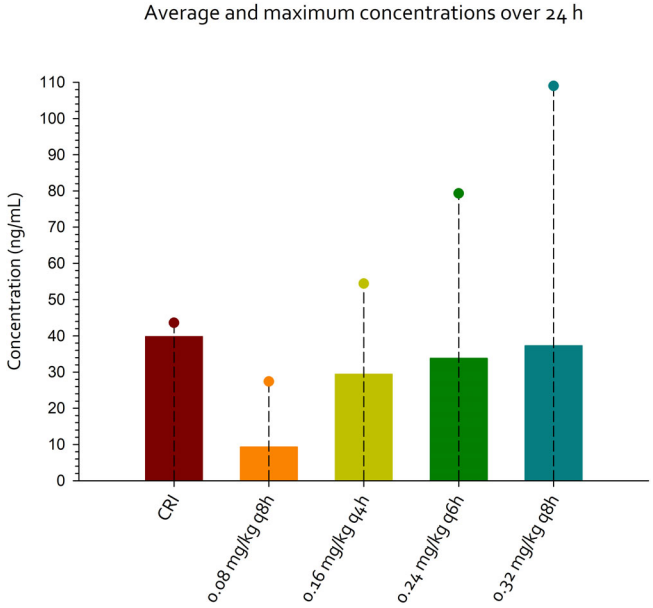

FIGURE 3 Average concentration (C_{avg}) measure for the intravenous continuous rate infusion (IV CRI) and simulated for each repeated subcutaneous (SC) bolus dosing regimens. The maximum concentration (C_{max}) measured for each is represented by the dot above the corresponding bar.

adverse effects and the fact that an IV bolus is not typically administered in equine clinical practice.

Since clinical administration of metoclopramide would require repeated SC bolus administration, rather than a single bolus, multiple dosing protocols were simulated to achieve the same total 24-h metoclopramide administration as the IV CRI (0.96 mg/kg). Each of these simulations showed a similar drug exposure to the IV CRI, as represented by the AUC. Previous studies suggest that the extrapyramidal effects of metoclopramide are concentration dependent,[3,4,12,16] so the higher C_{max} achieved by the simulated SC bolus protocols may increase the risk for these effects. The 0.16 mg/kg SC bolus every 4 h protocol had the closest C_{max} (54.4 ng/mL) to the measured IV CRI (43.6 ± 9.97 ng/mL), which may indicate that it carries the lowest risk of side effects of the simulated doses. This protocol had the highest accumulation index (1.45) of any of the simulated doses, meaning that the minimum (or trough) concentrations of metoclopramide remain higher in this protocol than the other simulated regimens due to the more frequent dosing strategy. The higher minimum concentrations may be associated with higher chance of metoclopramide toxicity, but it is unknown how this may factor into the risk of side effects. Additionally, the frequent administration schedule may decrease user compliance based on administrator availability and tolerance of patients to frequent, repeated injections. The 0.24 mg/kg SC bolus every 6 h protocol had a higher C_{max} (79.3 ng/mL) than either the 0.16 mg/kg SC bolus every 4 h or the IV CRI, which may mean it carries a higher risk of side effects than these

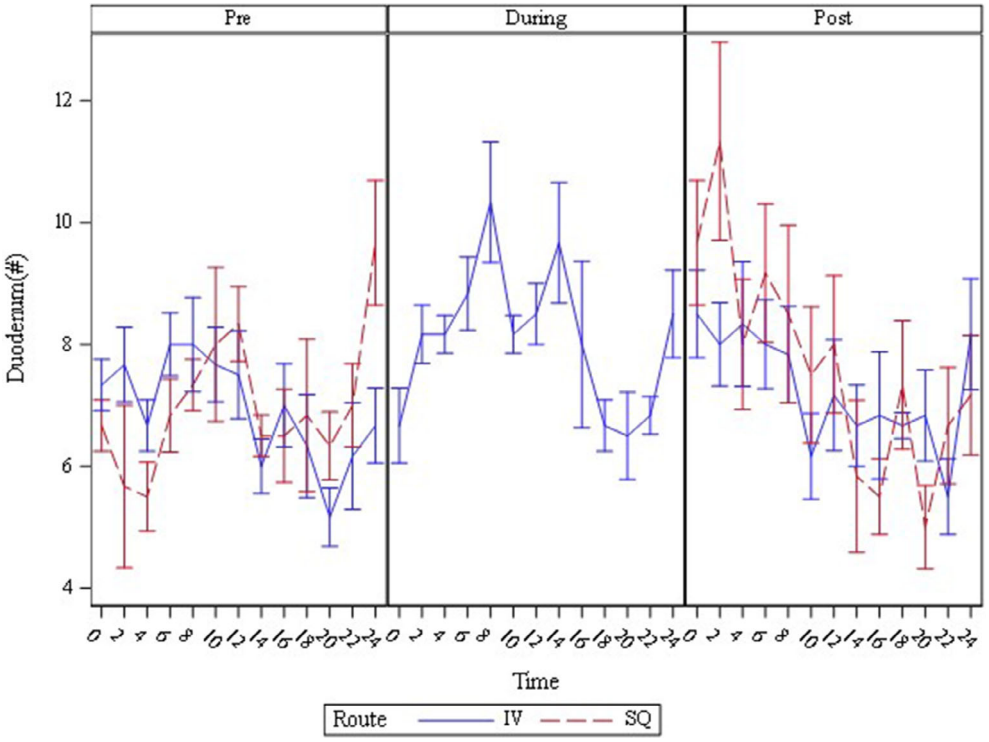

FIGURE 4 Mean number of duodenal contractions recorded at each time point during the intravenous continuous rate infusion (IV CRI) (blue solid line) and following the subcutaneous (SC) bolus (red dashed line). The left section ("Pre") represents the number of contractions recorded every 2 h prior to initiation of any treatment protocol. The middle section ("During") represents the number of contractions recorded every 2 h while the IV CRI was being administered (no equivalent time point exists for the SC bolus, as this was not "maintained" in the same way as the CRI. The right section ("Post") represents the number of contractions recorded every 2 h following discontinuation of the IV infusion or following drug administration in the case of the SC group.

regimens. The 0.32 mg/kg SC bolus every 8 h protocol had the closest average concentration of metoclopramide (C_{avg}; 37.3 ng/mL) to the IV CRI (39.8 ± 8.48 ng/mL) of any of the simulated doses, which may mean that it would be the most clinically equivalent to the prokinetic effects seen with the IV CRI. Unfortunately, this regimen had a C_{max} (109 ng/mL) that was more than double that of the IV CRI (43.6 ± 9.97 ng/mL), leading to the assumption that this protocol may carry a much higher risk of extrapyramidal effects than the other simulated SC bolus regimens or IV CRI.

While a few timepoints were determined to carry statistically significant differences in gastrointestinal motility based on duodenal ultrasonography and borborygmi auscultation, there were no obvious trends to provide information for clinical application. The raw data revealed very wide variations in the numbers of duodenal contractions and borborygmi across the horses in each group and within the same horse from one predrug administration monitoring period to the next. This could be due to the inherent variability in gastrointestinal motility in normal horses,[7] or to the insensitivity of the monitoring methods used. Ultrasonography was used as a semi-quantitative measure of small intestinal motility. Most other noninvasive markers of gastrointestinal motility, such as fecal output weight and recovery of ingested beads, are more closely related to and influenced by large intestinal motility and carry their own drawbacks, such as bead recovery failure.[7,21,24–26] Borborygmi auscultation is an insensitive measure of small intestinal motility, but it has been widely used in a variety of other studies as an assessment of motility.[7,21,24] Given the minimal number of statistically significant findings and the wide variation in the raw data, it was deemed likely that the noninvasive monitoring methods employed in this study were too insensitive to effectively monitor motility or draw conclusions regarding clinical trends.

No adverse side effects were noted in any horse, in either treatment group and there were no behavioral differences when compared to evaluations prior to drug administration. The lack of extrapyramidal effects during the IV CRI could mean that this administration protocol is not so fraught with complications as to warrant the need for SC bolus dosing to minimize the risk of adverse effects. The lack of side effects could also be attributable to the small sample size and use of systemically healthy

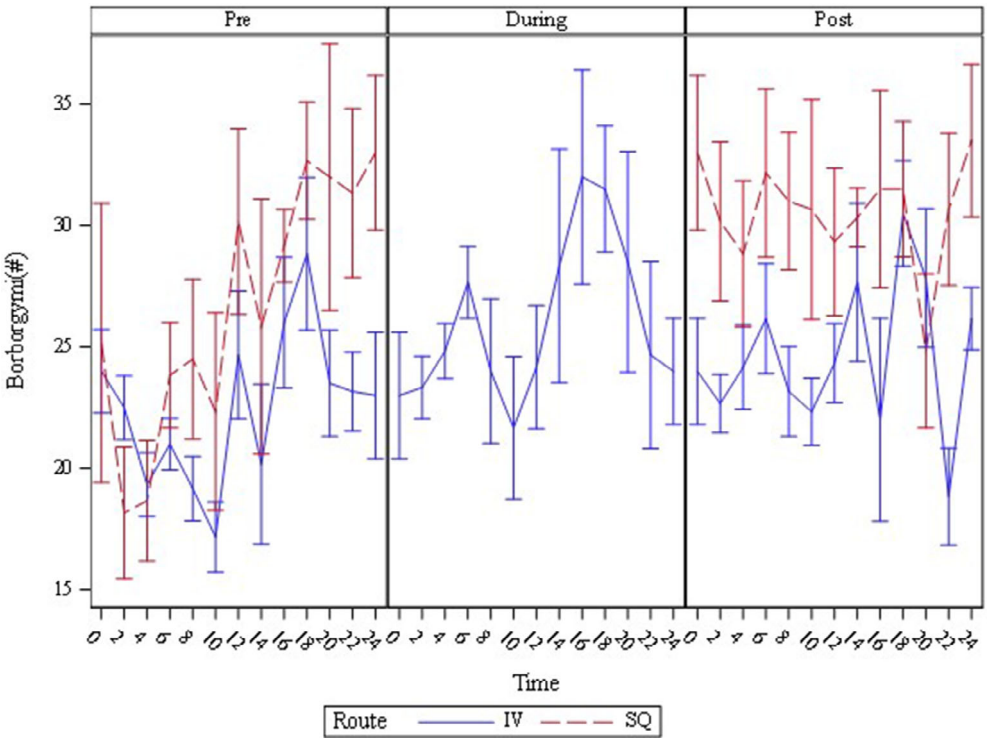

FIGURE 5 Mean number of borborygmi auscultated at each time point prior to, during, and after discontinuation of the intravenous continuous rate infusion (IV CRI) (blue solid line) and prior to and following the subcutaneous (SC) bolus (red dashed line). The left section ("Pre") represents the number of borborygmi recorded every 2 h prior to initiation of any treatment protocol. The middle section ("During") represents the number of borborygmi recorded every 2 h while the IV CRI was being administered (no equivalent time point exists for the SC bolus, as this was not "maintained" in the same way as the CRI. The right section ("Post") represents the number of borborygmi recorded every 2 h following discontinuation of the IV infusion or following drug administration in the case of the SC group.

horses. Regardless of the risk of extrapyramidal effects, SC bolus administration still carries the benefits of not needing to place and maintain an intravenous catheter, use additional equipment like a CRI pump, or to closely monitor ongoing drug administration.

During the course of the study, drug administration complications were encountered, which resulted in a minor method modification as detailed above. While the fluid pumps completed infusion of the IV CRI early in the first two horses to undergo that protocol (one pump ended 19 min short of the 24-h mark, the other ended 32 min short), all blood samples were labeled appropriately with collection times in relation to drug administration (23 h and 41 min and 23 h and 28 min, respectively, as opposed to 24 h). This allowed these samples to be accurately interpreted in context of the timing of drug administration and discontinuation and yield valid pharmacokinetic results. An additional complication was noted during IV CRI administration when one horse broke the IV CRI fluid line twice over the course of administration. Both times, video footage review facilitated identification of when exactly how long the IV CRI line was disconnected from the horse. This knowledge allowed accurate interpretation of the PK results obtained from this horse's samples following these incidents. Given the exact documentation of timings of disruption in drug delivery and application of those timings in PK analysis, these disruptions were not believed to negatively impact PK analysis.

Additional limitations of the study included a small sample size, the use of horses believed to be systemically healthy, the lack of a sham group, and lack of investigator blinding during assessment of GI motility. An additional limitation with respect to the simulated dosing strategies was the assumption that the drug followed linear elimination kinetics. The study was initially designed as a pharmacokinetic evaluation of the two dosing strategies, and, as such, a sample size of six was chosen. Given there is so little data regarding clinical use of metoclopramide, the decision was made to include monitoring for both motility and adverse effects. While a common sample size for pharmacokinetic studies, this sample size likely limited our ability to identify effects on motility and/or the incidence of adverse effects, leaving room for type 2 error with respect to this portion of the study. With respect to the limitation of using healthy horses, it is plausible that the pharmacokinetics, efficacy, and side

effect profile of metoclopramide may be altered in horses with clinical disease and/or those who are receiving other drugs concurrently, limiting the ability to extrapolate these data to clinical cases. Additionally, no bloodwork analyses were performed, which would have been beneficial to further characterize these horses as healthy as metoclopramide is metabolized in the liver and excreted in the urine. Avenues for further study would include further characterization of the multiple SC bolus dosing strategies, as well as a large-scale, prospective clinical trial evaluating the use of IV CRI versus SC protocols in clinical cases. While a true sham group was not used so as to avoid subjecting additional horses to invasive procedures, an attempt was made to use each horse as their own control for the GI motility and behavioral monitoring components of the study. For the 24 h prior to initiation of each treatment protocol, each horse was subjected to the same GI motility and behavioral monitoring that was to be performed during the treatment periods. The 24 h of monitoring prior to initiation of a treatment protocol was then utilized as control data for the sake of statistical comparison. In order to limit the impact of recent travel on these monitoring parameters, the horses were given a day to adjust to the hospital environment prior to initiation of the 24-h monitoring period that was used as the self-control. An additional limitation in the evaluation of GI function was the decision to evaluate motility without blinding for treatment. All auscultations and ultrasound examinations were therefore performed by a member of the study team who knew what treatment protocol the horse was undergoing. An additional limitation was the fact that auscultations were performed by multiple different investigators. Each investigator underwent a training period (provided by the primary author) to ensure that all investigators scored auscultatory findings the same way based on the criteria described in the methods in an attempt to minimize any interobserver variations.

In conclusion, based on the present findings, the 0.08 mg/kg administered as repeated SC boluses every 8 h is insufficient to result in drug exposure equivalent to that achieved with the 0.04 mg/kg/h IV CRI. If the widely accepted dosing strategy of 0.04 mg/kg/h via IV CRI is presumed to be efficacious, this particular SC bolus dosing protocol is considered unlikely to result in comparable clinical effects. Higher and/or more frequent SC bolus dosing protocols would be needed to achieve a similar drug exposure and thus presumably equivalent efficacy to the IV CRI. As SC bolus administration was demonstrated to be highly bioavailable and no adverse behavioral side effects were noted, it is a desirable avenue for further investigation. However, none of the six studied horses were seen to exhibit any adverse effects from the standard IV CRI, suggesting this administration method is likely well tolerated, albeit less convenient.

AUTHOR CONTRIBUTIONS

Brandon AB, DVM: Contributed to the study design, sample acquisition, review of recorded data, data interpretation, and drafted and revised the manuscript. Williams JM, DVM, MS, PhD, DACVS (Large Animal), DACVECC (Large Animal): Contributed to the study design, data interpretation, and manuscript preparation. Davis JL, DVM, PhD, DACVIM (Large Animal), DACVCP: Contributed to the study design, performed pharmacokinetic analysis and data interpretation, and contributed to manuscript preparation. Martin EM, DVM, PhD, DACVS (Large Animal): Contributed to the study design manuscript preparation. Capper AM, DVM: Contributed to sample acquisition and manuscript preparation. Crabtree NE, DVM, MS, DACVS (Large Animal), DACVECC (Large Animal): Contributed to the study design, sample acquisition, data interpretation, and manuscript preparation. All authors provided a critical review of the manuscript and endorse the final version. All authors are aware of their respective contributions and have confidence in the integrity of all contributions. Keys DA, PhD: Provided statistical analysis of the data. Dias B, DVM: Contributed to sample acquisition. Cox D, DVM: Contributed to sample acquisition. Council-Troche RM, MS: Performed method development and analytical analysis of samples.

FUNDING INFORMATION

This study was funded internally by the University of Georgia.

CONFLICT OF INTEREST STATEMENT

There are no financial or other conflicts of interest of any author.

REFERENCES

1. Damen S. Equine gastro-intestinal motility – a review on prokinetics, spasmolytics and their receptors. 2016 [Master's thesis; University of Ghent].
2. Van Hoogmoed LM, Nieto JE, Snyder JR, et al. Survey of prokinetic use in horses with gastrointestinal injury. *Vet Surg*. 2004; 33:279-285.
3. Agass RF, Brennan M, Rendle DI. Extrapyramidal side effects following subcutaneous metoclopramide injection for the treatment of post operative ileus. *Equine Vet Educ*. 2017;29(10): 564-568.
4. Dart AJ, Peauroi JR, Hodgson DR, et al. Efficacy of metoclopramide for treatment of ileus in horses following small intestinal surgery: 70 cases (1989–1992). *Aust Vet J*. 1996;74(4):280-284.
5. Epstein K, Short D, Parente E, Reef V, Southwood L. Serial gastrointestinal ultrasonography following exploratory celiotomy

in normal adult ponies. *Vet Radiol Ultrasound*. 2008;49:584-588.
6. Freeman DE, Hammock P, Baker GJ, et al. Short-and long-term survival and prevalence of postoperative ileus after small intestinal surgery in the horse. *Equine Vet J*. 2000;32(32):42-51.
7. Gerring EEL, Hunt JM. Pathophysiology of equine postoperative ileus: effect of adrenergic blockade, parasympathetic stimulation and metoclopramide in an experimental model. *Equine Vet J*. 1986;18:249-255.
8. Lawson AL, Sherlock CE, Mair TS. Equine duodenal motility, assessed by ultrasonography, as a predictor of reflux and survival following colic surgery. *Equine vet Educ*. 2021;33(2):84-89.
9. Lefebvre D, Hudson NPH, Elce YA, et al. Clinical features and management of equine post operative ileus (POI): survey of diplomates of the American colleges of veterinary internal medicine (ACVIM), veterinary surgeons (ACVS) and veterinary emergency and critical care (ACVECC). *Equine Vet J*. 2016;48:714-719.
10. Beder NA, Mourad AA, Aly MA. Ultrasonographic evaluation of the effects of the administration of neostigmine and metoclopramide on duodenal, cecal, and colonic contractility in Arabian horses: a comparative study. *Vet World*. 2020;13(11):2447-2451.
11. Delsalle C, Lefebvre RA, Schuurkes JAJ, et al. Gastro-intestinal motility in horses: a practical overview of the therapeutic use of prokinetic agents. *Vlaams Diergeneeskd Tijdschr*. 2006;75:122-139.
12. Hunt JM, Gerring EL. A preliminary study of the effects of metoclopramide on equine gut activity. *J vet Pharmacol Therap*. 1986;9:109-112.
13. Lefebvre D, Pirie RS, Handel IG, Tremaine WH, Hudson NPH. Clinical features and management of equine post operative ileus: survey of diplomates of the European colleges of equine internal medicine (ECEIM) and veterinary surgeons (ECVS). *Equine Vet J*. 2016;48:182-187.
14. Nieto JE, Rakestraw PC, Snyder JR, Vatistas NJ. In vitro effects of erythromycin, lidocaine, and metoclopramide on smooth muscle from the pyloric antrum, proximal portion of the duodenum and middle portion of the jejunum of horses. *Am J Vet Res*. 2000;61:413-419.
15. Okamura K, Sasaki N, Yamada M, Yamada H, Inokuma H. Effects of mosapride citrate, metoclopramide hydrochloride, lidocaine hydrochlorides, and cisapride citrate on equine gastric emptying, small intestinal and caecal motility. *Res Vet Sci*. 2009;86:302-308.
16. Sojka JE, Adams SB, Lamar CH, et al. Effect of butorphanol, pentazocine, meperidine, or metoclopramide on intestinal motility in female ponies. *Am J Vet Res*. 1988;49(4):527-529.
17. Lee HW, Ji HY, Kim HY, Park ES, Lee KC, Lee HS. Determination of metoclopramide in human plasma using hydrophilic interaction chromatography with tandem mass spectrometry. *J Chromatogr B*. 2009;877(18–19):1716-1720.
18. Shah VP, Midha KK, Dighe S, et al. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. *Pharm Res*. 1992;9:588-592.
19. Gomaa N, Uhlig A, Schusser GF. Effect of Buscopan compositum on the motility of the duodenum, cecum and left ventral colon in healthy conscious horses. *Berl Munch Tierarztl Wochenschr*. 2011;124(3–4):168-174.
20. Mitchell CF, Malone ED, Sage AM, Niksich K. Evaluation of gastrointestinal activity in healthy horses using B mode and doppler ultrasonography. *Can Vet J*. 2005;46(2):134-140.
21. Patton ME, Leise BS, Baker RE, Andrews FM. The effects of bit chewing on borborygmi, duodenal motility, and gastrointestinal transit time in clinically normal horses. *Vet Surg*. 2022;51:88-96.
22. VanderBroek AR, Reef VB, Aitken MR, et al. Assessing gastrointestinal motility in healthy horses comparing auscultation, ultrasonography and an acoustic gastrointestinal surveillance biosensor: a randomised, blinded, controlled crossover proof of principle study. *Equine Vet J*. 2019;51:246-251.
23. Grimsrud KN, Mama KR, Steffey EP, Stanley SD. Pharmacokinetics and pharmacodynamics of intravenous medetomidine in the horse. *Vet Anaesth Analg*. 2012;39:38-48.
24. Boscan P, Van Hoogmoed LM, Farver TB, Snyder JR. Evaluation of the effects of the opioid agonist morphine on gastrointestinal tract function in horses. *Am J Vet Res*. 2006;67(6):992-997.
25. Elfenbein JR, Robertson SA, Corser AA, Urion RJ, Sanchez LC. Systemic effects of a prolonged continuous infusion of ketamine in healthy horses. *J Vet Intern Med*. 2011;25(5):1134-1137.
26. King JN, Gerring EL. The action of low dose endotoxin on equine bowel motility. *Equine Vet J*. 1991;23(1):11-17.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Brandon AM, Williams JM, Davis JL, Martin EG, Capper AM, Crabtree NE. Evaluation of pharmacokinetics of metoclopramide administered via subcutaneous bolus and intravenous constant rate infusion to adult horses. *Veterinary Surgery*. 2024;53(6):1111-1122. doi:10.1111/vsu.14128