Title

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Journal

Veterinary anaesthesia and analgesia, 47(5)

ISSN

1467-2987

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Publication Date

2020-09-01

DOI

10.1016/j.vaa.2020.04.004

Peer reviewed

HHS Public Access

Author manuscript

Vet Anaesth Analg. Author manuscript; available in PMC 2021 September 01.

Published in final edited form as:

Vet Anaesth Analg. 2020 September ; 47(5): 694–704. doi:10.1016/j.vaa.2020.04.004.

Metabolism, pharmacokinetics and selected pharmacodynamic effects of codeine following a single oral administration to horses

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Abstract

Objective—To describe the pharmacokinetics and selected pharmacodynamic variables of codeine and its metabolites in Thoroughbred horses following a single oral administration.

Study design—Prospective experimental study.

Animals—A total of 12 Thoroughbred horses, nine geldings and three mares, aged 4–8 years.

Methods—Horses were administered codeine (0.6 mg kg^{-1}) orally and blood was collected before administration and at various times until 120 hours post administration. Plasma and urine samples were collected and analyzed for codeine and its metabolites by liquid chromatography-mass spectrometry, and plasma pharmacokinetics were determined. Heart rate and rhythm, step counts, packed cell volume and total plasma protein were measured before and 4 hours after administration.

Results—Codeine was rapidly converted to the metabolites norcodeine, codeine-6-glucuronide (C6G), morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Plasma codeine concentrations were best represented using a two-compartment model. The C_{max}, t_{max} and elimination $t_{½}$ were 270.7 ± 136.0 ng mL^{-1}, 0.438 ± 0.156 hours and 2.00 ± 0.534 hours, respectively. M3G was the main metabolite detected (C_{max} 492.7 ± 35.5 ng mL^{-1}), followed by C6G (C_{max} 96.1 ± 33.8 ng mL^{-1}) and M6G (C_{max} 22.3 ± 4.96 ng mL^{-1}). Morphine and norcodeine were the least abundant metabolites with C_{max} of 3.17 ± 0.95 and 1.42 ± 0.79 ng mL^{-1}, respectively. No significant adverse or excitatory effects were observed.

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Authors' contributions

SRG and DSM: study execution, data analysis, manuscript preparation. CJF: study design, final review of manuscript. PHK: data analysis, manuscript preparation. HKK: study design, study execution, data analysis, manuscript preparation. All authors read and approved the final version of the manuscript.

Conflict of interest statement

The authors declare no conflict of interest

Conclusions and clinical relevance—Following oral administration, codeine is rapidly metabolized to morphine, M3G, M6G, C6G and norcodeine in horses. Plasma concentrations of M6G, a presumed active metabolite of morphine, were comparable to concentrations reported previously following administration of an analgesic dose of morphine to horses. Codeine was well tolerated based on pharmacodynamic variables and behavioral observations.

Keywords

codeine; horse; metabolism; pharmacodynamics; pharmacokinetics

Introduction

Codeine is a naturally occurring opioid commonly used in humans to treat mild to moderate pain or as a cough suppressant (Fuller & Jackson 1990; Barnett 2001). In veterinary medicine, codeine has been used successfully as an antitussive agent and to manage postoperative and cancer pain in dogs and cats (Gaynor 2008; KuKanich 2010; Martins et al. 2010; Poliacek et al. 2017). The five primary metabolites of codeine have been identified in humans (Chen et al. 1991; Williams et al. 2001). O-demethylation of codeine generates morphine that is subsequently conjugated via uridine 5′-diphospho-glucuronsyltransferases to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). N-demethylation of codeine produces norcodeine, and direct glucuronidation of codeine generates codeine-6-glucuronide (C6G), the primary metabolite in humans (Chen et al. 1991; Williams et al. 2001; Dean 2012; Fig. 1).

The analgesic effects of codeine may be primarily derived from the generation of morphine, which has 200 times greater affinity for the μ-opioid receptor than codeine (Sindrup & Brøsen 1995; Madadi & Koren 2008). This mechanism is supported by reports of increased analgesia in a subset of the human population characterized as extensive metabolizers in whom plasma concentrations of morphine are high following codeine administration (Sindrup et al. 1992). Furthermore, M6G, a morphine metabolite, has analgesic properties greater than morphine in rats, mice and humans (Frances et al. 1992; Stain et al. 1995; Grace & Fee 1996; Lötsch & Geisslinger 2001). C6G has a similar affinity for the μ-opioid receptor as codeine and was shown to be antinociceptive in rats, suggesting it may also contribute to analgesia resulting from codeine administration (Srinivasan et al. 1997).

There are currently a limited number of therapeutic options available for the treatment of pain in horses; therefore, additional pharmacologic agents that are efficacious for moderate to severe pain without extensive adverse effects are warranted. Non-steroidal anti-inflammatory drugs are frequently used and generally well tolerated but are associated with increased risk of gastrointestinal ulcers and renal toxicity in susceptible individuals (Martínez Aranzales et al. 2014; Knych 2017). Commonly prescribed opioids in human medicine, such as morphine, may produce dose-dependent neuroexcitation and gastrointestinal stasis in horses, thereby limiting their use in this species (Combie et al. 1981; Knych et al. 2014; Hamamoto-Hardman et al. 2019). As a prodrug of morphine and other metabolites with analgesic potential, codeine may prove to be an effective analgesic for horses. Activation of prodrugs through biotransformation processes is a rate-limiting

process. The slower appearance of morphine in the systemic circulation following codeine administration may limit the adverse effects seen with administration of morphine.

The metabolism and pharmacokinetics of codeine have not been reported in horses. Accordingly, the objective of this study was to describe the pharmacokinetics and selected pharmacodynamic properties of orally administered codeine and its metabolites in horses as a first step in characterizing this drug as a potential therapeutic agent. We hypothesized that the metabolic profile of codeine would be similar to that described in other species, including production of the analgesic metabolites morphine and M6G.

Materials and methods

Animals

A total of 12 University-owned adult Thoroughbred horses, nine geldings and three mares, aged 4–8 years and weighing, mean ± standard deviation, 524.8 ± 49.3 kg were used for this study. The horses were routinely exercised 5 days per week according to established laboratory protocols (Corado et al. 2017) that continued throughout the study with the exception of the first day. During the study, horses were housed in stalls at the Veterinary Medicine Teaching Hospital and were potentially subject to environmental stimuli. Prior to the start of the study, the horses were determined to be healthy by physical examination, complete blood count and serum biochemistry panel. No medications were administered for at least 2 weeks prior to starting the study. This study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis, CA, USA (no. 20319).

Drug administration and instrumentation

Drug administration for all 12 horses occurred on the same day. Each horse was weighed immediately prior to administration of a single oral dose of codeine sulfate (0.6 mg kg^{-1}; 30 mg codeine tablets; West-Ward Pharmaceuticals Corp., NJ, USA). Codeine tablets were dissolved in 6 mL of water in the dosing syringe and delivered directly into the oral cavity. Subsequently, an additional 6 mL of water was added to the dosing syringe and delivered in the same manner. On day 1 of the study, a 14 gauge, 13.3 cm catheter (BD, ON, Canada) was placed aseptically into one jugular vein for blood collection.

On the day of the study, two Step Monitors (StepWatch Version 3; Modus Health llc, WA, USA) were attached to nine of the 12 horses (number limited by availability of Step Monitors) set to count the number of steps per minute. Step Monitors were attached to the distal left thoracic and right pelvic limbs using Velcro straps. Polo wraps were applied to all four limbs to decrease the likelihood of the animal favoring one limb. Step counts were recorded starting a minimum of 30 minutes prior to dosing and continued until 4 hours post administration. Step count data was imported using the commercial StepWatch software Version 3.4 (Modus Health llc) and the number of steps averaged over 10 minute intervals.

All 12 horses were fitted with Holter monitors (Trillium 5000; Forest Medical llc, NY, USA) for continual monitoring of heart rate (HR) and rhythm recorded over the same time span as the Step Monitors.

Monitoring behavior

Horses were observed continuously for behavioral changes for the first 4 hours after drug administration and at each blood sampling time thereafter. Monitoring was performed by equine technicians involved in the routine handling of these horses and familiar with the individual animal's behavior. Observations included changes in temperament (including but not limited to irritation, restlessness, anxiety, excitation, depression) or physical activity (pacing, rolling, kicking and pawing). Additionally, the frequency of defecation, number of fecal balls and consistency of feces were observed and recorded hourly for 24 hours post drug administration.

Sample collection

Blood samples were collected immediately prior to drug administration and at 15, 30 and 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 18, 24, 30, 36, 48, 72, 96 and 120 hours post drug administration. Prior to each sample collection, 10 mL of blood was drawn from the catheter and discarded. Blood (20 mL) was aspirated from the catheter and transferred into two 10 mL ethylenediaminetetraacetic acid (EDTA) collection tubes (Covidien, MA, USA). Following sample collection, the catheter was flushed with 10 mL of heparinized saline (100 IU mL^{-1}). The catheter was removed after the 24 hour sample collection and the remaining six samples were collected via direct venipuncture into EDTA-containing blood tubes. Blood samples were placed on ice and centrifuged at 3000 ***g*** for 10 minutes within an hour of collection. Plasma was transferred to cryovials (Phoenix Research Products, NC, USA) and stored at −20 °C until analysis.

An aliquot from each blood sample was taken for determination of packed cell volume (PCV) and total plasma protein (TP) prior to drug administration and at 15, 30 and 45 minutes, and 1, 2, 4, 5 and 8 hours following drug administration. PCV and TP were measured by microhematocrit and refractometer, respectively. Measurements were made in duplicate and the average recorded.

Urine samples were collected at approximately 24, 48, 72, 96 and 120 hours post drug administration by free catch. The horses were trained to urinate in response to the sound of a whistle. The time of collection was recorded and samples were stored at −20 °C until analysis. All samples were collected within 1 hour of the actual time point.

Determination of drug concentration

The analytical reference standards for codeine, C6G, norcodeine, morphine, M3G, M6G and the internal standards d6-morphine, d6-codeine and d3-M6G were obtained from Cerilliant Corporation, TX, USA. High performance liquid chromatography grade or better solvents were purchased from the following manufacturers: acetonitrile (ACN) and water from Burdick & Jackson, MI, USA; methanol and buffer reagents from Thermo Fisher Scientific, NJ, USA; and formic Acid, 98%, from Sigma-Aldrich, MO, USA.

Plasma sample analysis

The six analytes were combined into a single working solution. The working standard solutions were diluted with drug-free equine plasma to reach concentrations ranging 0.1–

1500 ng mL^{-1} to serve as the plasma calibrators. Calibration curves for all analytes (0.1–1500 ng mL^{-1}) and negative control samples were prepared using drug-free equine plasma and were prepared fresh for each quantitative assay. Quality control samples (equine plasma fortified with analyte at three concentrations within the standard curve) were included with each sample.

Samples were prepared using protein precipitation as described previously (Knych et al. 2014). The internal standards, d6-morphine (50 ng mL^{-1}), d6-codeine (25 ng mL^{-1}) and d3-M6G (25 ng mL^{-1}) (Cerilliant Corporation) were added to all samples and 30 μL injected into the liquid chromatography tandem-mass spectrometry (LC-MS/MS) system.

The analyte concentrations were measured in equine plasma using a TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Fisher Scientific, CA, USA) equipped with 1100 series liquid chromatography systems (Agilent Technologies Inc., CA, USA). The spray voltage was 4000 V, the vaporizer temperature was 200 °C and the sheath and auxiliary gas were 50 and 30, respectively (arbitrary units). The product masses and collision energies of each analyte were optimized by infusing the standards into the TSQ Quantum Ultra. Chromatography employed a Zorbax Eclipse-XDB-Phenyl 3 × 100 mm, 3 μm column (Agilent Technologies Inc.) and a linear gradient of ACN in water with a constant 0.2% formic acid at a flow rate of 0.45 mL $minute^{-1}$. The initial ACN concentration was held at 5% for 0.3 minutes, ramped to 40% over 4.7 minutes, then ramped to 90% over 0.3 minutes before re-equilibrating for 3.8 minutes at initial conditions.

Detection and quantification was conducted using selective reaction monitoring of initial precursor ion for codeine [mass to charge ratio ($m\,z^{-1}$) 300.1], C6G ($m\,z^{-1}$ 176.2), norcodeine ($m\,z^{-1}$ 286.1), morphine ($m\,z^{-1}$ 286.1), M3G ($m\,z^{-1}$ 462.1), M6G ($m\,z^{-1}$ 462.1) and the internal standards d6-codeine ($m\,z^{-1}$ 306.1), d6-morphine ($m\,z^{-1}$ 292) and d3-M6G ($m\,z^{-1}$ 465.1). The response for the product ions for codeine ($m\,z^{-1}$ 165, 215, 199), C6G ($m\,z^{-1}$ 300, 215), norcodeine ($m\,z^{-1}$ 165, 152), morphine ($m\,z^{-1}$ 165, 152), M3G ($m\,z^{-1}$ 165, 286), M6G ($m\,z^{-1}$ 165, 286) and the internal standards d6-codeine ($m\,z^{-1}$ 165), d6-morphine ($m\,z^{-1}$ 165), d3-M6G ($m\,z^{-1}$ 289) were plotted and the appropriate peaks at the proper retention time were integrated using Quanbrowser software (Thermo Fisher Scientific). Quanbrowser software was used to generate the calibration curves and quantitate codeine, C6G, norcodeine, morphine, M3G and M6G in all samples using linear regression analysis with a weighting factor of 1/X used for all calibration curves.

Urine sample analysis

Urine calibrators were prepared by dilution of the working standard solutions with drug-free equine urine to concentrations ranging 0.25–4000 ng mL^{-1}. Quality control samples (equine urine fortified with analyte at three concentrations within the standard curve, except M3G that had four concentrations) were included with each sample set as an additional check of accuracy.

Prior to analysis, 1 mL of urine was diluted with 100 μL of water and the internal standards added (d6-morphine, d6-codeine and d3-M6G internal standard at 0.25 ng μL^{-1}). Solid phase extraction was performed using CUC18 3cc 200mg Clean-Up Extraction Columns

(United Chemical Technologies Inc., PA, USA) per the manufacturer's instructions. The samples were dried under nitrogen at 45 °C and reconstituted in 150 μL of 5% ACN in water with 0.2% formic acid, and 20 μL was injected into the LC-MS/MS system. Detection and quantification were the same as described above, except the product ion used for quantitation for codeine was ($m\,z^{-1}$ 215).

Pharmacokinetic analysis

Pharmacokinetic parameters were determined for orally administered codeine by compartmental analysis using Phoenix WinNonlin Version 8.2 (Certara, NJ, USA). The model of best fit and the appropriate error model were determined by visual analysis of concentration and residual plots and coefficients of variation, Schwarz Bayesian criteria and Akaike information criterion. Noncompartmental analysis was used to determine the pharmacokinetic parameters for the metabolites morphine, M3G, M6G, C6G and norcodeine.

Statistical analysis

Statistical analyses using Stata/IC Version 13.1 (StataCorp LP, TX, USA) were used to determine significant differences in pharmacodynamic variables between baseline and each time point. Data were analyzed using a mixed-effects analysis of variance, with the horse as the random effect and time and dose as the fixed effects. *Post hoc* comparisons were performed with a Bonferroni multiple comparison adjustment to preserve a nominal significance level of 0.05.

Results

Plasma concentration determination and pharmacokinetic analysis

The intraday, interday, analyst-to-analyst precision and accuracy of the assay were determined by assaying quality control samples in replicates ($n = 6$) for codeine, C6G, norcodeine, morphine, M3G and M6G. Accuracy was reported as percent nominal concentration, and precision was reported as percent relative standard deviation (Tables 1 & 2). The responses for codeine, C6G, norcodeine, morphine, M3G and M6G were linear and gave correlation coefficients of 0.99 or better. The technique was optimized to provide a limit of quantitation (LOQ) of 0.1 ng mL^{-1} for codeine, C6G, norcodeine, morphine and M3G, and 0.25 ng mL^{-1} for M6G. The limit of detection (LOD) was approximately 0.05 ng mL^{-1} for codeine, C6G, norcodeine, morphine and M3G, and 0.15 ng mL^{-1} for M6G.

Codeine and the five metabolites norcodeine, C6G, morphine, M3G and M6G were all detected in plasma following oral administration. Plasma concentration-time curves for codeine and its metabolites are shown in Figure 2, and plasma concentrations in Table 3. A two-compartment extravascular model parameterized based on clearance and with an additive + multiplicative error model provided the best fit for the plasma codeine concentration data:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-kat}$$

where C_p is plasma concentration, A is coefficient for distribution phase, α is distribution rate constant, t is time, B is coefficient for elimination phase, β is the first elimination rate constant, C is coefficient for absorption phase and ka is absorption rate constant.

The pharmacokinetic parameters for codeine are listed in Table 4 and parameters generated for the codeine metabolites using noncompartmental analysis are listed in Table 5.

All five codeine metabolites were detected at levels above the LOD in plasma at the first time point (15 minutes post dose). Of the 12 horses, two horses at 8 hours post drug administration and three horses by 12 hours had codeine plasma concentrations below the LOQ. Furthermore, codeine plasma concentrations were below the LOQ in nine horses by 18 hours and in all horses by 24 hours. Plasma concentrations for M3G fell below the LOQ in 11 horses by 120 hours. M6G concentrations were below the LOQ in eight horses at 30 hours and all horses by 36 hours. Morphine was below the LOQ in 11 horses by 48 hours and all horses by 72 hours. C6G concentrations were below the LOQ in all horses by 48 hours. Norcodeine was detected in plasma for the shortest amount of time with concentrations below the LOQ in all horses by 18 hours.

Urine concentration determination

Of the 12 horses, codeine was <LOQ in 11 horses at 24 hours post drug administration (Table 6). All five metabolites were detected in the urine of all horses (Table 6). Norcodeine concentrations were <LOQ in all horses by 48 hours and C6G concentrations were <LOQ in six horses at 48 hours and in nine horses at 72 hours. Morphine concentrations were <LOQ in five horses at 72 hours, and M6G concentrations were <LOQ in six horses at 72 hours. M3G concentrations were >LOQ at the last measured time point (120 hours post drug administration) in all horses studied.

Physiologic and behavioral responses

Overall, codeine was well tolerated by all horses in that no adverse behavioral changes were noted. Formed feces were noted regularly and fecal output remained consistent throughout the study period for all horses. HR data were not collected from one horse because of a technical failure with the Holter monitor. HR was increased and the percentage of atrioventricular blocks decreased relative to baseline at 2.5, 3 and 4 hours post administration (all $p < 0.001$; Fig. 3) but remained within accepted normal range. Step counts were increased relative to baseline at 30 minutes ($p = 0.001$), 2.5 ($p = 0.005$) and 3 hours ($p = 0.003$) after dosing (Fig. 4). PCV and TP were increased at 4, 6 and 8 hours (all $p < 0.001$; Table 7).

Discussion

The primary objective of this study was to characterize the pharmacokinetics of codeine in horses and to identify and quantitate metabolites. In horses, as has been reported for humans, codeine undergoes extensive biotransformation, generating five primary metabolites: norcodeine, C6G, morphine, M3G, and M6G (Hidetoshi et al. 1970). In the current study, the primary metabolites identified in plasma were M3G, C6G and M6G, with lower concentrations of morphine and norcodeine detected. This is in contrast to humans and dogs

in which C6G is the predominant metabolite in plasma and relatively low concentrations of morphine and morphine glucuronides are detected (Findlay et al. 1986; Chen et al. 1991; KuKanich 2010). Whereas codeine was only detected in the urine of one horse at 24 hours, high concentrations of M3G as well as moderate concentrations of C6G, M6G and morphine were detected in all horses. This is similar to reports by Yeh & Woods (1971) that identified M3G as the major metabolite in rat urine. However, in contrast to the current report, in rats only low concentrations of C6G were detected and M6G was not detected in the urine. In humans, C6G was the main metabolite detected in the urine after oral administration, followed by parent compound, with much lower concentrations of morphine conjugates, morphine and norcodeine (Chen et al. 1991). Similarly, C6G is reported to be the main urinary metabolite in both guinea pigs and rabbits following subcutaneous administration of codeine; however, only rabbits had significant concentrations of M3G in addition to C6G (Oguri et al. 1990). Compared with other species, it appears that the horse rapidly metabolizes codeine to morphine and extensively glucuronidates both morphine and codeine substrates.

In the present study, codeine plasma concentrations following a single oral administration were best fit using a two-compartment model. Similar to humans, codeine was readily absorbed from the gastrointestinal tract and rapidly metabolized with all five metabolites detected in the plasma at the first time point measured (Guay et al. 1987). The t_{max} in horses was 0.44 ± 0.16 hours compared with 0.6 ± 0.2 hours in humans administered 60 mg oral codeine (Guay et al. 1987) and 0.91 hours in dogs administered codeine (1.43 mg kg^{-1}) orally (KuKanich 2010). Maximum concentrations in the current study were 270.7 ± 136.0 ng mL^{-1}, much greater than the 10.0 ng mL^{-1} observed in dogs when a higher dose was administered (1.43 mg kg^{-1}; KuKanich 2010). The fairly rapid 2.00 ± 0.53 hour terminal elimination half-life of codeine is similar to that reported in dogs (1.6 hours; KuKanich 2010) and shorter than that in humans (3.6–4.8 hours; Kirchheiner et al. 2007).

In evaluating codeine as a potential opioid analgesic for use in horses, the concentrations of morphine and the active morphine metabolite M6G are of particular interest as they have been postulated to contribute to the analgesic effect of codeine in humans (Shimomura et al. 1971; Yoshimura et al. 1973; Sindrup et al. 1992; Poulsen et al. 1996). M6G is a μ-opioid agonist, similar to morphine, but has been shown to be a more potent analgesic than the parent compound in humans, mice and rats (Shimomura et al. 1971; Paul et al. 1989; Stain et al. 1995; Grace & Fee 1996). Morphine (0.2 mg kg^{-1}) has been suggested to be the minimum analgesic dose in horses (Sande et al. 2017). In the current study, oral administration of codeine (0.6 mg kg^{-1}) resulted in comparable plasma concentrations of M6G to that previously reported following morphine (0.2 mg kg^{-1}) administered IV to horses (Knych et al. 2014; Hamamoto-Hardman et al. 2019). The low concentrations of morphine coupled with higher concentrations of M6G in the plasma following oral codeine administration suggest that it is rapidly metabolized to morphine and subsequently conjugated to M6G. Despite the low levels of morphine observed in plasma, if M6G is ultimately shown to have analgesic properties as in other species, it is possible that M6G concentrations may be sufficient to provide clinically relevant analgesia in horses following codeine administration. Further studies describing the analgesic effects of both codeine and M6G in horses are warranted.

In humans, biotransformation of codeine to morphine is a result of cytochrome P450 2D6. Cytochrome P450 2D6 is highly polymorphic (Sindrup & Brøsen 1995) and genetic variations in the CYP2D6 gene result in increased (ultra-rapid metabolizers) or decreased (poor metabolizers) enzymatic activity. As codeine is metabolized to morphine by CYP2D6 and morphine is believed to provide most of the analgesia associated with codeine administration (Findlay et al. 1978; Sindrup & Brøsen 1995), individuals who are poor and ultra-rapid metabolizers may experience therapeutic failure (lack of analgesia) or toxicity (morphine overdose), respectively. In horses, a member of the CYP2D family (CYP2D82; Knych et al. 2019) is also responsible for the biotransformation of codeine to morphine. Furthermore, researchers have speculated that polymorphisms in genes coding for members of the CYP2D family may also exist in horses (Corado et al. 2016); therefore, it is important to note that polymorphisms in these genes could impact the therapeutic efficacy of codeine in horses. Among humans, the prevalence of CYP2D6 polymorphisms varies greatly among ethnic groups (Kirchheiner et al. 2007). Similarly, it is possible that such differences may be observed between different breeds of horses. The use of a single breed of horse in this study represents a limitation in the assessment of codeine metabolism, and future studies using additional breeds are warranted.

A secondary objective of the present study was to describe selected pharmacodynamic (behavioral and physiologic) responses following oral administration of codeine to horses. Morphine and other μ-agonist opioids result in dose-dependent excitation and increased locomotor activity in horses (Mama et al. 1992; Clutton 2010). In the present study, however, excitation was not observed following codeine administration. Although step counts were significantly increased at 30 minutes, 2.5 and 3 hours after dosing, standard deviations were large. It is possible that the increased locomotor activity was related to external environmental stimuli rather than responses to the drug or metabolites. The increases in HR were minimal but may have a similar origin as no correlation with peak drug/metabolite concentrations is apparent. PCV and TP were unchanged until 4–8 hours when both statistically increased; however, they remained within normal ranges for horses (Tasker 1966).

Codeine, at the dose administered in the present study, evoked no visible excitatory response in the horses. There were no signs of colic, and fecal output remained consistent throughout the study periods. Limitations of this study include the lack of auscultation of intestinal borborygmi, which would provide further information on the effect of codeine on gastrointestinal motility. Additionally, the absence of a non-treated control group and step count data for two horses as well as the uncontrolled external environment the horses were exposed to limit the conclusions that can be drawn from the pharmacodynamic data. Further studies addressing these areas of the study design are necessary to thoroughly assess the pharmacodynamic effects of codeine. This study focused on behavioral and physiologic effects of oral administration of codeine to horses as an initial evaluation. Before codeine can be recommended for the treatment of pain in clinical cases, additional studies assessing its effects on thermal and/or mechanical nociception are necessary.

Conclusion

Codeine (0.6 mg kg^{-1}) was administered orally to 12 horses and plasma concentrations of codeine and metabolites analyzed up to 5 days. The results indicate that codeine is rapidly and extensively metabolized to morphine, M3G, M6G, C6G and norcodeine following oral administration in the horse. Plasma concentrations of the presumed active metabolite, M6G, were comparable to previous reports of morphine metabolism in horses. Whereas studies of analgesic efficacy are necessary, the lack of obvious adverse effects coupled with generation of reportedly analgesic concentrations of morphine and M6G are encouraging for further study of the use of codeine in horses.

Acknowledgements

This project was supported by the Center for Equine Health with funds provided by the State of California pari-mutuel fund and contributions by private donors and the California Horse Racing Board. The authors acknowledge Kelsey Seminoff, Stacy Steinmetz, Kirsten Kanarr, Briana Hamamoto, Sandy Yim and Russell Baden for their assistance with this project.

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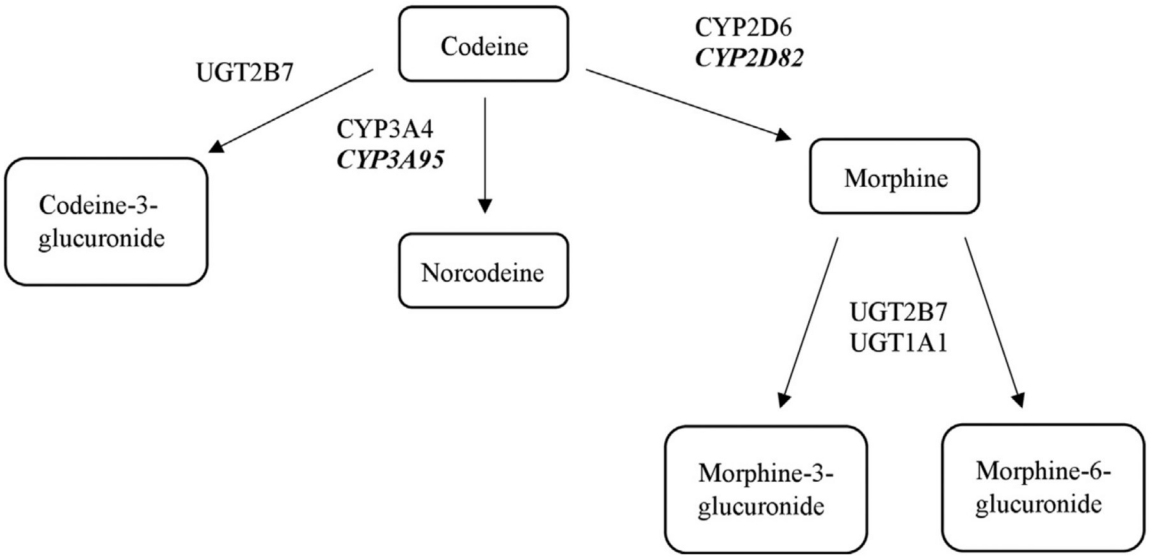

Figure 1.
Metabolic pathway of codeine in horses. Enzymes in humans are in regular font, enzymes in horses in bold italic.

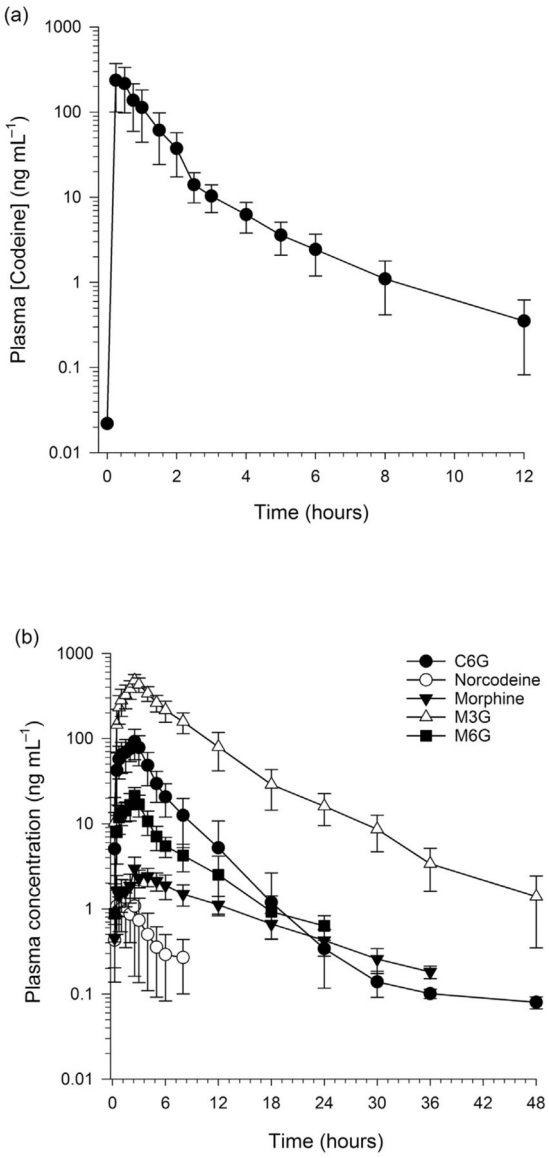

Figure 2.

Average plasma concentrations of (a) codeine and (b) codeine metabolites with respect to time after oral administration of codeine (0.6 mg kg^{-1}) in 12 exercised Thoroughbred horses.

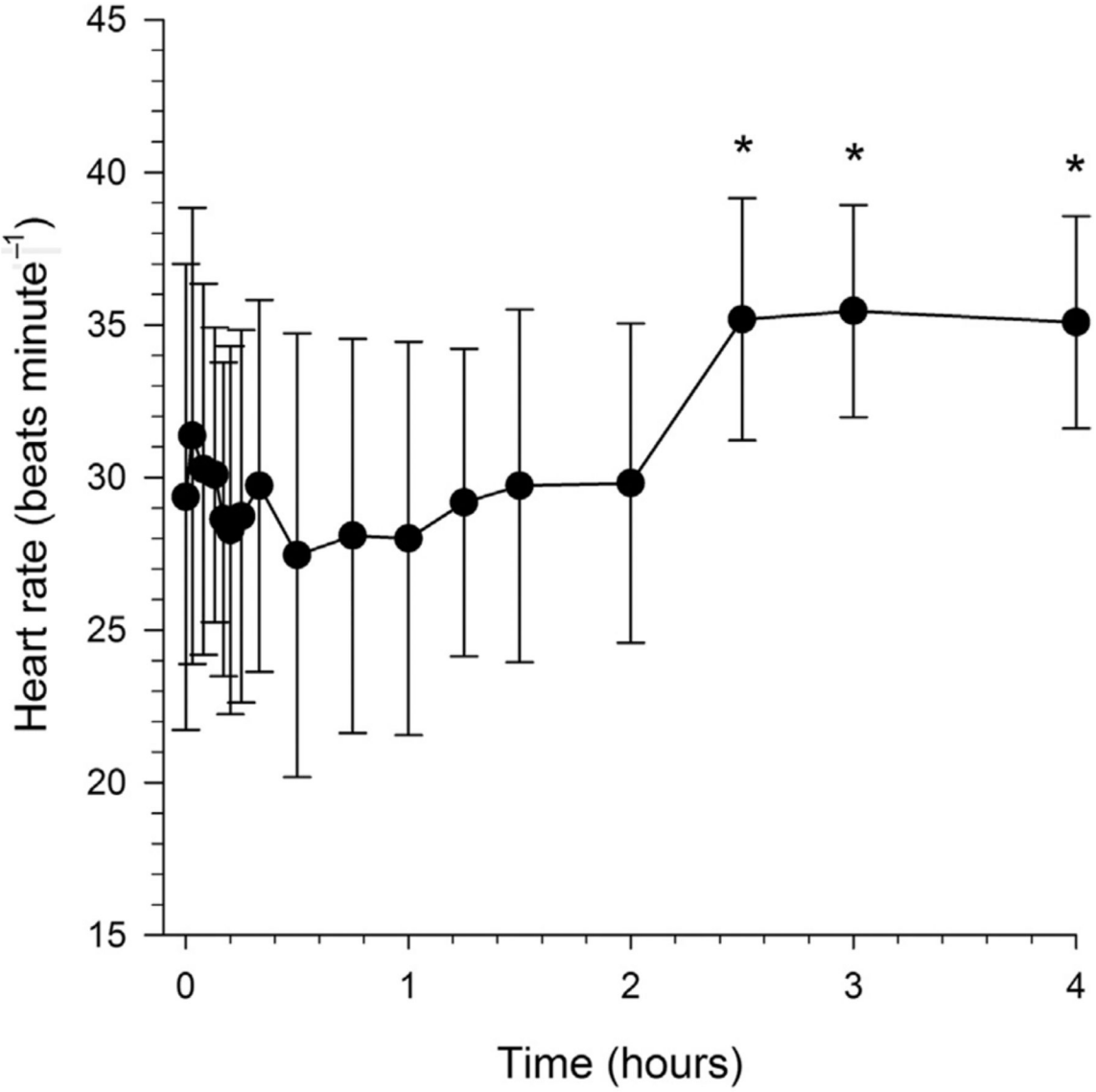

Figure 3.

Heart rate (mean ± standard deviation) before (baseline, time 0) and following a single oral administration of codeine (0.6 mg kg^{-1}) in 12 exercised Thoroughbred horses.

*Significantly different from baseline ($p < 0.05$).

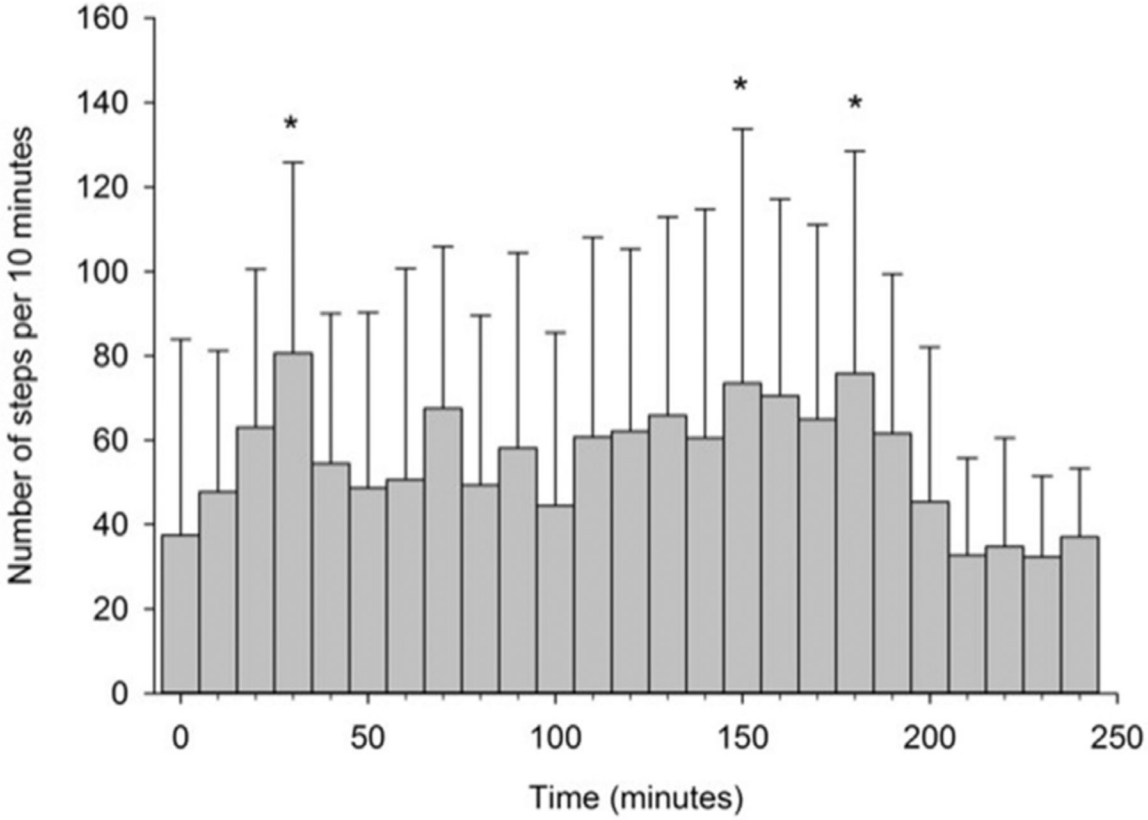

Figure 4.

Number of steps (mean ± standard deviation) before (baseline, time 0) and following a single oral administration of codeine (0.6 mg kg^{-1}) to nine exercised Thoroughbred horses. The number of steps recorded from the left thoracic and right pelvic limbs from individual horses were averaged at each time and the number of steps summed over 10 minute increments. *Significantly different from baseline ($p < 0.05$).

Table 1

Intra- and interday accuracy (reported as the percent nominal concentration) and precision [reported as percent relative standard deviation (SD)] values for liquid chromatography-tandem mass spectrometry analysis of codeine and the metabolites norcodeine, codeine-6-glucuronide (C6G), morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) in equine plasma.

Analyte	Concentration (ng mL^{-1})	Intraday accuracy (% nominal concentration)	Intraday precision (% relative SD)	Interday accuracy (% nominal concentration)	Interday precision (% relative SD)
Codeine					
	0.75	96.0	3.0	100	3.0
	100	100	2.0	99.0	2.0
	800	92.0	2.0	95.0	3.0
Norcodeine					
	0.75	90.0	4.0	94.0	5.0
	100	90.0	2.0	92.0	2.0
	800	91.0	3.0	94.0	4.0
C6G					
	0.75	98.0	8.0	7.0	6.0
	100	92.0	2.0	2.0	3.0
	800	91.0	3.0	3.0	4.0
Morphine					
	0.75	110	2.0	108	5.0
	100	101	2.0	105	2.0
	800	94.0	2.0	97.0	3.0
M3G					
	0.75	89.0	7.0	91.0	5.0
	100	100	4.0	107	4.0
	800	97.0	4.0	101	3.0
M6G					
	0.75	96.0	3.0	97.0	5.0
	100	104	3.0	107	3.0
	800	97.0	3.0	100	2.0

Table 2

Intra- and interday accuracy (reported as the percent nominal concentration) and precision [reported as percent relative standard deviation (SD)] values for liquid chromatography-tandem mass spectrometry analysis of codeine and the metabolites norcodeine, codeine-6-glucuronide (C6G), morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) in equine urine.

Analyte	Concentration (ng mL^{-1})	Intraday accuracy (% nominal concentration)	Intraday precision (% relative SD)	Interday accuracy (% nominal concentration)	Interday precision (% relative SD)
Codeine					
	1.5	97.0	6.0	98.0	5.0
	7.5	95.0	3.0	101	4.0
	250	104	4.0	102	5.0
Norcodeine					
	1.5	95.0	6.0	94.0	5.0
	7.5	99.0	4.0	100	5.0
	250	101	4.0	102	5.0
C6G					
	1.5	94.0	9.0	100	9.0
	7.5	96.0	5.0	96.0	6.0
	250	94.0	6.0	94.0	6.0
Morphine					
	1.5	1	7.0	101	6.0
	7.5	100	4.0	101	4.0
	250	101	4.0	99.0	4.0
M3G					
	1.5	102	10.0	101	9.0
	7.5	112	8.0	114	6.0
	250	105	7.0	105	5.0
	2000	100	10.0	99.0	10.0
M6G					
	1.5	94.0	8.0	95.0	7.0
	7.5	97.0	4.0	102	4.0
	250	105	5.0	103	4.0

Table 3

Plasma concentrations (mean ± standard deviation) of codeine, norcodeine, codeine-6-glucuronide (C6G), morphine, morphine-3-glucuoronide (M3G) and morphine-6-glucuronide (M6G) following a single oral dose of codeine (0.6 mg kg^{-1}) in 12 exercised Thoroughbred horses.

Time (hours)	Concentration (ng mL^{-1})					
	Codeine	**Norcodeine**	**C6G**	**Morphine**	**M3G**	**M6G**
0.25	236.28 ± 135.72	0.43 ± 0.23	5.05 ± 8.49	1.02 ± 0.31	10.32 ± 9.91	2.15 ± 1.60
0.5	216.57 ± 118.55	1.07 ± 0.57	42.19 ± 25.49	1.73 ± 0.82	145.57 ± 75.23	7.92 ± 4.56
0.75	137.28 ± 77.75	0.96 ± 0.42	57.25 ± 23.87	1.43 ± 0.38	232.78 ± 85.55	11.87 ± 4.34
1	113.30 ± 68.96	0.99 ± 0.46	64.31 ± 25.53	1.56 ± 0.65	280.58 ± 98.61	13.50 ± 3.99
1.5	61.20 ± 37.00	0.94 ± 0.59	68.58 ± 28.52	1.62 ± 0.57	322.80 ± 99.94	14.26 ± 3.74
2	37.36 ± 20.07	0.87 ± 0.47	75.46 ± 21.93	1.86 ± 0.69	383.07 ± 94.54	16.69 ± 5.93
2.5	14.03 ± 5.44	1.08 ± 0.91	91.94 ± 35.68	2.97 ± 1.08	481.22 ± 81.42	21.34 ± 5.15
3	10.31 ± 3.70	0.73 ± 0.60	77.94 ± 30.27	2.30 ± 0.54	429.17 ± 82.09	16.84 ± 4.84
4	6.23 ± 2.43	0.50 ± 0.39	48.22 ± 20.32	2.40 ± 0.59	338.52 ± 65.44	10.67 ± 3.39
5	3.59 ± 1.51	0.35 ± 0.26	29.50 ± 11.75	2.12 ± 0.51	262.29 ± 57.49	7.06 ± 2.26
6	2.44 ± 1.25	0.29 ± 0.21	20.67 ± 8.67	1.89 ± 0.60	214.85 ± 59.66	5.46 ± 1.46
8	1.10 ± 0.68	0.27 ± 0.17	12.54 ± 7.30	1.49 ± 0.42	156.94 ± 42.92	4.24 ± 1.51
12	0.35 ± 0.27	0.17 ± 0.00	5.20 ± 5.57	1.19 ± 0.25	79.68 ± 38.02	2.51 ± 1.64
18	0.21 ± 0.03	ND	2.15 ± 1.95	0.88 ± 0.11	28.78 ± 14.35	1.20 ± 0.47
24	<LOQ - ND	ND	0.34 ± 0.22	0.43 ± 0.15	16.05 ± 6.55	0.82 ± 0.10
30	<LOQ - ND	ND	0.14 ± 0.05	0.26 ± 0.08	8.62 ± 3.93	0.46 ± 0.10
36	ND	ND	<LOQ - ND	0.18 ± 0.03	3.38 ± 1.77	ND
48	ND	ND	<LOQ - ND	0.15 ± 0.00	1.87 ± 0.95	ND
72	ND	ND	<LOQ - ND	ND	0.26 ± 0.08	ND
96	ND	ND	<LOQ - ND	ND	0.14 ± 0.02	ND
120	ND	ND	ND	ND	0.11 ± 0.00	ND

LOQ, limit of quantification; ND, not determined.

Table 4

Pharmacokinetic parameters for codeine after a single oral administration of codeine (0.6 mg kg^{-1}) to 12 exercised Thoroughbred horses. Parameters were generated using compartmental analysis.

Parameter	Mean ± standard deviation
K_a ($hour^{-1}$)	19.3 ± 30.9
CL/F (mL $minute^{-1}$ kg^{-1})	45.9 ± 31.9
CL_2/F (mL $minute^{-1}$ kg^{-1})	6.98 ± 5.68
V/F (L kg^{-1})	2.07 ± 1.93
V_2/F (L kg^{-1})	0.916 ± 0.613
C_{max} (ng mL^{-1})	270.7 ± 136.0
t_{max} (hour)	0.438 ± 0.156
AUC (ng hour mL^{-1})	286.5 ± 129.4
A (ng mL^{-1})	534.9 ± 359.4
B (ng mL^{-1})	20.7 ± 9.53
α ($hour^{-1}$)	1.76 ± 0.324
β ($hour^{-1}$)	0.365 ± 0.079
$t_{½\alpha}$ (hour)	0.407 ± 0.077
$t_{½\beta}$ (hour)	2.00 ± 0.534

A and B, intercepts for t = 0 for the model equation and α and β are the slopes for the model equation; $Cp = Ae^{-\alpha t} + Be^{-bt} + Ce^{-kat}$; AUC, area under the plasma-time curve; C_{max}, maximum measured plasma concentration; CL/F and CL_2/F, clearance corrected for bioavailability from the central and second compartments, respectively; K_a, absorption rate constant; $t_{½\alpha}$, distribution half-life; $t_{½\beta}$, elimination half-life; t_{max}, time at which maximum concentration is reached; V/F, volume of distribution for the central compartment; V_2/F, volume of distribution for the second compartment.

Table 5

Pharmacokinetic parameters (mean ± standard deviation) for codeine metabolites after a single oral administration of codeine (0.6 mg kg^{-1}) to 12 exercised Thoroughbred horses. Parameters were generated using noncompartmental analysis.

Parameter	C6G	Norcodeine	Morphine	M3G	M6G
C_{max} (ng mL^{-1})	96.1 ± 33.8	1.36 ± 0.78	3.17 ± 0.950	492.7 ± 85.5	22.3 ± 4.96
t_{max} (hour)	2.23 ± 0.67	1.17 ± 0.82	1.96 ± 0.891	2.42 ± 0.359	2.23 ± 0.670
t_{last} (hour)	32.0 ± 4.01	6.25 ± 2.45	32.0 ± 6.93	72.0 ± 22.9	24.0 ± 5.12
AUC_{inf} (ng hour mL^{-1})	409.7 ± 158.9	4.79 ± 3.41	34.0 ± 8.06	3290 ± 694.5	112.1 ± 30.1
λ_Z ($hour^{-1}$)	0.186 ± 0.047	0.358 ± 0.153	0.087 ± 0.011	0.100 ± 0.033	0.135 ± 0.053
Half-life λ_Z (hour)	4.00 ± 1.27	2.24 ± 0.872	8.08 ± 1.00	7.58 ± 2.41	6.28 ± 3.48

AUC_{inf}, area under the curve extrapolated to infinity; C_{max}, maximum measured serum concentration; C6G, codeine-6-glucuronide; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; t_{max}, time of maximum serum concentration; t_{last}, time of the last measured concentration above LOQ; λ_Z, slope of the terminal elimination curve; half-life λ_Z, terminal half-life.

Table 6

Urine concentrations (mean ± standard deviation) of codeine, norcodeine, codeine-6-glucuronide (C6G), morphine, morphine-3-glucuoronide (M3G) and morphine-6-glucuronide (M6G) following a single oral dose of codeine (0.6 mg kg^{-1}) to 12 exercised Thoroughbred horses.

Time (hour)	Concentration (ng mL^{-1})					
	Codeine	Norcodeine	C6G	Morphine	M3G	M6G
24	0.55 ± 0.00 (*n* = 1)	1.27 ± 0.84	29.1 ± 18.3	52.5 ± 41.4	1714.0 ± 1021.0	50.7 ± 39.4
48	ND	ND	1.47 ± 1.38	2.67 ± 2.85	137.6 ± 130.8	4.31 ± 5.32
72	ND	ND	1.01 ± 0.62	0.56 ± 0.27	21.8 ± 18.3	0.83 ± 0.54
96	ND	ND	1.38 ± 0.0 (*n* = 1)	ND	5.71 ± 4.40	0.43 ± 0.04
120	ND	ND	ND	ND	3.88 ± 2.29	0.38 ± 0.0 (*n* = 1)

ND, not detected.

Table 7

Packed cell volume (PCV) and total plasma protein (TP), mean ± standard deviation, before (baseline) and after oral administration of codeine (0.6 mg kg^{-1}) to 12 exercised Thoroughbred horses.

Time (hour)	PCV (%)	TP (g 100 mL^{-1})
Baseline	31.9 ± 2.8	6.0 ± 0.2
0.25	31.6 ± 2.8	6.1 ± 0.2
0.5	31.3 ± 2.8	6.1 ± 0.2
0.75	31.3 ± 3.0	5.9 ± 0.2 *
1	31.4 ± 3.1	6.0 ± 0.3
2	31.2 ± 3.5	6.1 ± 0.3
4	39.7 ± 2.9 *	7.0 ± 0.3 *
6	39.1 ± 2.9 *	6.8 ± 0.4 *
8	36.6 ± 1.8 *	6.3 ± 0.3 *

* Significantly different from baseline ($p < 0.05$).