RESEARCH PAPER

Pharmacokinetic-pharmacodynamic modelling of intravenous buprenorphine in conscious horses

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Abstract

Objective Describe the pharmacokinetics of buprenorphine and norbuprenorphine in horses and to relate the plasma buprenorphine concentration to the pharmacodynamic effects.

Study design Single phase non-blinded study.

Animals Six dedicated research horses, aged 3–10 years and weighing 480–515 kg.

Methods Thermal and mechanical nociceptive thresholds, heart and respiratory rates and locomotor activity were measured before and at 15, 30, 45 & 60 minutes and 2, 4, 6, 8, 12 & 24 hours post-administration of 10 µg kg⁻¹ buprenorphine IV. Intestinal motility was measured 1, 6, 12 & 24 hours after buprenorphine administration. Venous blood samples were obtained before administration of buprenorphine 10 µg kg⁻¹ IV and 1, 2, 4, 6, 10, 15, 30, 45 & 60 minutes and 2, 4, 6, 8, 12 & 24 hours afterwards. Plasma buprenorphine and norbuprenorphine concentrations were measured using a liquid chromatography-tandem mass spectroscopy (LC-MS/MS) assay with solid-phase extraction. A non-compartmental method was used for analysis of the plasma concentration–time data and plasma buprenorphine concentrations were modelled against two dynamic effects (change in thermal threshold and mechanical threshold) using a simple Emax model.

Results Plasma buprenorphine concentrations were detectable to 480 minutes in all horses and to 720 minutes in two out of six horses. Norbuprenorphine was not detected. Thermal thresholds increased from 15 minutes post-buprenorphine administration until the 8–12 hour time points. The increase in mechanical threshold ranged from 3.5 to 6.0 Newtons (median: 4.4 N); and was associated with plasma buprenorphine concentrations in the range 0.34–2.45 ng mL⁻¹.

Conclusions and clinical relevance The suitability of the use of buprenorphine for peri-operative analgesia in the horse is supported by the present study.

Keywords equine, mechanical nociceptive threshold, opioid, pain, thermal nociceptive threshold.

Introduction

Buprenorphine is a partial µ opioid agonist that has been used extensively to provide both intra- and post-operative analgesia in man and a number of animal species (Conzemius et al. 1994; Roughan & Flecknell 2002; Dahan et al. 2006; Steagall et al. 2009a). It has recently gained a UK Marketing Authorisation for administration to horses for analgesia at a dose of up to 10 µg kg⁻¹ intravenously (IV). Previous studies have indicated that buprenorphine (at this dose) administered in combination with acepromazine produces antinociception to a
thermal stimulus for approximately 9 hours (Love et al. 2012). In a very recent study, buprenorphine 10 μg kg⁻¹ provided near comprehensive analgesia for 24 hours in ponies undergoing castration (Love et al. 2013).

A few published papers describe the pharmacokinetics of buprenorphine in horses (Seino et al. 2003; Messenger et al. 2011; Davis et al. 2012), but none have evaluated concentration-effect data. The aim of this study was therefore to describe the pharmacokinetics of buprenorphine and its metabolite norbuprenorphine in horses, and to relate the plasma buprenorphine concentration to the pharmacodynamic effects, particularly antinociception.

**Materials and methods**

**Animals**

Six Thoroughbred horses, aged 3–10 years and weighing 480–515 kg, were used in this ‘non-blinded’ single phase study. Horses were kept at grass and were brought into individual pens, comprising both an indoor and outdoor area, at least 24 hours prior to experimental procedures. Hay was removed the night prior to treatment but water was freely available at all times. Horses were assessed as healthy based on physical examination, a complete blood count and serum biochemistry. No sedatives or analgesics were administered to the animals within three weeks of the start of this study. All assessments and blood sampling were performed by the same investigator. The study was conducted under UK Home Office Project Licence Number 30/2420.

**Drug dosage and administration**

The horses were weighed using an electronic weighbridge on the day of the experiment and fly repellent was applied to the body of the horse. Local anaesthetic cream (EMLA Cream 5%; Astra Zeneca UK Ltd. UK) was applied to the skin over both jugular veins following clipping of hair. Two IV catheters were placed and secured with tissue adhesive; an 18 gauge catheter was inserted into the right jugular vein and a 14 gauge catheter was inserted into the left jugular vein. Following collection of baseline blood samples and measurements each horse received 10 μg kg⁻¹ buprenorphine over 30 seconds (Vetergesic Multidose, Alstoe Animal Health, Sherriff Hutton, UK) through the 18 gauge catheter.

**Measurements**

Thermal and mechanical thresholds were measured using apparatus described previously (Chambers et al. 1993 (mechanical), Love et al. 2012 (thermal)). In brief the thermal testing equipment consisted of a 1 cm² probe comprising a small heating element and a thermistor (Topcat Metrology Ltd, UK). This was applied to a clipped area of skin over the horses’ withers. The starting skin temperature was recorded and the probe activated via a remote controlled handset so that heating started at 0.5 °C second⁻¹. A skin twitch signified the endpoint, heating was stopped and the threshold temperature recorded. If no response occurred heating stopped at a ‘cut-out’ of 53 °C to prevent skin damage. Mechanical thresholds were measured using an actuator cuff attached to a control box via 3 mm internal diameter PVC tubing (Custom built for the School of Veterinary Sciences). The cuff was positioned with the upper edge 4 cm distal to the carpus so that a 1.5 mm blunt ended pin was in contact with the dorsal aspect of the third metacarpal bone. The force used to drive the pin against the leg was increased at a constant rate until the horse lifted his leg, signifying the end-point. A ‘cut-out’ of 15 N was included in the equipment design. On a separate occasion control data were collected from the same horses with respect to mechanical (n = 6 horses) and thermal (n = 5 horses) thresholds after IV injection of 5% glucose. The collection and analysis of these data are reported elsewhere (Love et al. 2012).

Locomotor activity was measured using a pedometer that was attached to a bandage on the forelimb to record the total number of steps throughout the investigation. On a separate occasion control data with respect to locomotor activity was collected from the same horses after IV injection of 5% glucose; the collection and analysis of these data are reported elsewhere (Love et al. 2012). In addition, prior to measurement of nociceptive thresholds, heart rate (HR) was determined by auscultation; respiratory rate (f_R) was measured by counting the number of thoracic excursions in 1 minute. Gastrointestinal motility was assessed by abdominal auscultation and the number of piles of faeces produced were counted at intervals after drug administration.
Baseline data were recorded as the mean of three measurements of each variable made at 15 minute intervals before treatment administration. Single measurements of thermal and mechanical nociceptive thresholds, HR and fR were made at 15, 30, 45 and 60 minutes and 2, 4, 6, 8, 12 and 24 hours post-buprenorphine administration. Locomotor activity was measured continuously using the pedometer and the number of steps taken in three different time periods after buprenorphine administration calculated. Gastrointestinal motility and the number of faecal piles produced were counted 1, 6, 12 and 24 hours after treatment administration. The highest environmental temperature was recorded daily using a digital thermometer (Checktemp1, Hanna Instruments, Mauritius).

Blood sampling

Blood samples were taken before treatment administration and 1, 2, 4, 6, 10, 15, 30, 45 and 60 minutes, and 2, 4, 6, 8, 12 and 24 hours after administration of buprenorphine. Blood samples were taken from the dedicated IV catheter inserted into the left jugular vein. Initially, 5 mL of blood was aspirated from the catheter into a heparinised syringe. The blood sample (10 mL) for analysis was then withdrawn from the catheter into a plain syringe and immediately transferred into a heparinised blood tube labelled with the horses’ identification number and the sample time. After the sample was obtained the catheter was flushed with 10 mL 0.9% saline.

Blood samples were stored on ice immediately following collection. Within two hours the samples were centrifuged for five minutes at 503 g. The plasma was removed from the sample tube using a pipette and split into three aliquots that were stored in separate freezers at −20 °C for safe keeping.

Sample analysis for plasma buprenorphine concentrations

Plasma samples were thawed and analysed for buprenorphine and norbuprenorphine concentrations using a liquid chromatography-tandem mass spectroscopy (LC-MS/MS) assay with solid-phase extraction as described by Steagall et al. (2013) in compliance with Good Laboratory Practice Regulations (Quotient Bioresearch Limited, Fordham, Cambridgeshire, UK). The Mass Spectrometer operated in positive ionization mode. Output was referenced to a calibration line over the range of 0.100–200 ng mL⁻¹ for buprenorphine and 0.500–200 ng mL⁻¹ for norbuprenorphine. Using this calibration plot, outputs were subjected to least squares regression analysis using a quadratic fit (weighted 1/x²) to provide values for correlation coefficient and back-calculated concentrations. Buprenorphine inter-batch accuracy (% Relative Error) ranged from −8.8% [high Quality Control (QC)] to 4.7% (low QC). Inter-batch precision error (% CV) was <7% (medium QC). Inter-batch precision for norbuprenorphine was 2.4% at the low QC level, 9.9% at the medium QC level and 1.7% at the high QC level.

Pharmacokinetic analysis

A non-compartmental method was used for analysis of the plasma concentration–time data (Benet & Galeazzi 1979). The elimination half life (t₁/₂el) was calculated using nonlinear least squares regression analysis of the concentration-time curve, and the areas under the curve (AUC) and its first moment (AUMC) were calculated by the linear trapezoidal rule to the final concentration-time point (Ct). From these values, the apparent volume of distribution at steady state (Vdss = dose × AUMC/AUC²), mean residence time (MRT = AUMC/AUC) and systemic clearance (Cl = dose/AUC) were determined.

Pharmacodynamic analysis

The pharmacodynamic end points evaluated were skin and rectal temperatures, thermal and mechanical thresholds, HR, fR, step counts, faecal production and gastrointestinal motility, which were measured at various time points to 24 hours.

Pharmacokinetic-pharmacodynamic modelling

PK and PK/PD modelling were carried out by non-linear regression (WinNonlin 5.2; Pharsight Corporation, CA, USA). Quality of fit was assessed by determination of the Akaike’s information criterion (Yamaoka et al. 1978) and by visual inspection of the residuals. For the PK analysis, individual plasma concentration-time courses were best characterised by a tri-exponential equation after weighing the datapoints by the inverse of the square-fitted value. Plasma buprenorphine concentrations were modelled against two dynamic effects (change in mechanical threshold and step counts) using an
indirect response model (Danieka et al. 1993). Modelling of the changes in thermal threshold was not possible due to the high number of measurements obtained at the cut out temperature. Indirect response models were written in WinNonlin to capture negative hysteresis and the response measured (R) results from factors controlling the development (K\textsubscript{in}) or the dissipation (K\textsubscript{out}) of R as in equation 1:

\[
\frac{dR}{dt} = K_{in} - K_{out} \times R 
\]  

(1)

where \( \frac{dR}{dt} \) is the rate of change of the response R over time. \( K_{in} \) is the first-order rate constant for development of the response and \( K_{out} \) the zero-order rate constant for dissipation of the response. The fitted baseline response, \( R_0 \), is the ratio between \( K_{in} \) and \( K_{out} \) and the models were subsequently parameterised as in equation 2:

\[
\frac{dR}{dt} = K_{in} - (K_{out}/R_0) \times R 
\]  

(2)

For mechanical threshold testing, the fitted baseline force (\( R_0 \), in Newton) was set by the equilibrium between the tolerance to the stimulus (\( K_{in} \), Newton hour\(^{-1} \)) and nociception (\( K_{out} \), in hour\(^{-1} \)). Increases in response (R) resulted either from attenuation of the stimulus (decrease in \( K_{in} \)) or moderation of nociception (decreased \( K_{out} \)). We elected that buprenorphine moderated nociception in a non-linear fashion (following a sigmoid \( I_{max} \) model) according to the following equation (equation 3):

\[
\frac{dR}{dt} = K_{in} - (K_{in}/R_0) \times \left( 1 - \frac{I_{max} \times C^n}{IC_{50}^n + C^n} \right) \times R 
\]  

(3)

where \( I_{max} \) is proportional to the maximal mechanical threshold (no unit), \( IC_{50} \) is the drug concentration (ng mL\(^{-1} \)) that would achieve 50% of the maximum threshold increase and \( n \) is the slope of the concentration-effect relationship (no unit).

For the locomotion endpoint, we assumed that the fitted baseline steps count \( R_0 \) (steps hour\(^{-1} \)) resulted from equilibrium between the motivation to move (\( K_{in} \), steps hour\(^{-1} \)) and calmness (\( K_{out} \), hour\(^{-1} \)). We elected that buprenorphine stimulated locomotion by reducing the central motor inhibition according to equation 4:

\[
\frac{dR}{dt} = K_{in} - (K_{in}/R_0) \times \left( 1 - \frac{C^n}{IC_{50}^n + C^n} \right) \times R 
\]  

(4)

with \( I_{max} \) set as 1 as there was no limit to stimulation of locomotion. \( IC_{50} \) is the drug concentration that would achieve 50% of the peak response and \( n \) is the slope. Control (responses to glucose from Love et al. 2012) and buprenorphine data were modelled simultaneously for both endpoints and placebo response was fitted to a constant value. As a result, the buprenorphine and control functions shared the same fitted baseline \( R_0 \) value.

The PK/PD modelling used individual PK parameters estimates to characterise plasma concentration (C, in ng mL\(^{-1} \)) along time in a two-stage approach. Step count data, but not mechanical threshold data, were weighted by the inverse of the fitted value. For each endpoint, a set of PD parameters (\( K_{in} \), \( IC_{50} \), \( R_0 \), \( n \) and \( I_{max} \) when appropriate) were estimated by the model. The PK/PD model allowed simulations of the effect of different doses for both endpoints, using arithmetic mean PD parameters (step count) or geometric mean PK parameters or PD parameters (mechanical threshold).

Statistical analyses

Statistical analyses were carried out using SPSS for Windows (SPSS 14.0, Chicago, IL, USA). Data were tested for normality using the Kolmogorov-Smirnov test and appropriate parametric or non-parametric statistical analyses were applied. Normally distributed data are presented as mean ± SD and non-normally distributed data as median (range). When thermal and mechanical thresholds exceeded the cut-off (thermal 53 °C, mechanical 15 N) the maximum value was recorded as the threshold value. Mean ± SD post-treatment HR and f\( R \) were calculated as a summary measure for each horse by averaging the data collected over the 24 hour observation period. Mean HR and f\( R \) before and after buprenorphine administration were compared using a paired samples \( t \)-test. Step count data following dosing with buprenorphine were recorded for three time periods, similar to those defined in a previous study (Love et al. 2012): first hour after dosing; between 1 and 8 hours after dosing; and between 8 and 24 hours. These step count data were compared with control step count data obtained on a separate occasion from the same horses following administration of 5% glucose (Love et al. 2012) using the Friedman Test. Significance was set at the 5% level.

Results

All horses completed the study without any significant adverse events.
Pharmacodynamic parameters

Skin temperature and thermal thresholds

Skin temperature increased above baseline in all horses from 15 minutes after buprenorphine administration; mean ± SD skin temperature peaked at 36.5 ± 2 °C 30 minutes after buprenorphine administration. Rectal temperature was measured in each horse whenever an increase in skin temperature was recorded and remained within the range 37–37.5 °C. Skin temperature had returned to the baseline values by the following morning (24 hour sample point). Thermal thresholds increased to the maximum temperature (cut out) of 53 °C from 15 minutes post-buprenorphine administration until the 8–12 hour time-points in most horses. Figure 1 shows the thermal threshold following buprenorphine against time in hours. The environmental temperatures recorded on each study day ranged between 18.4 °C and 27.2 °C (mean ± SD 21.8 ± 3.1 °C).

Mechanical thresholds

Mechanical thresholds increased after buprenorphine administration for a variable period of time in each horse as shown in Fig. 2. The peak effect was seen at time points between 45 and 240 minutes (median 60 minutes).

Heart and respiratory rates

The measured HR and fR remained within clinically acceptable limits throughout the study period. The mean ± SD heart rates before, 34 ± 3 beats min-
ute minute⁻¹, and after, 43 ± 2 beats minute⁻¹, buprenorphine administration, were statistically significantly different (p = 0.001). The peak increase in HR was observed between 15 and 240 minutes after dosing. There was no significant effect of buprenorphine on the f0, which was 12 ± 2 and 12 ± 1 breaths per minute before and after buprenorphine administration, respectively.

Step counts
Complete data were available for step counts following administration of 5% glucose and buprenorphine (10 µg kg⁻¹) and these are shown in Table 1.

After IV buprenorphine there was a significant overall increase in the steps count in period 2 compared with period 1 (p = 0.032); however this can be attributed to the different duration of measurement (1 hour compared to 6 hours for periods 1 and 2, respectively). The step count hour⁻¹ in period 3 was lower than the step count (per hour) in periods 1 and 2. When compared with the change in step counts observed following administration of glucose to the same horses (Love et al. 2012) there was a significant increase in the number of steps during each time period in all horses when receiving buprenorphine; as well as in the total number of steps taken to 24 hours post-treatment (p = 0.043).

Faecal production and gastrointestinal motility
The numbers of piles of faeces produced by each horse in the 24 hours post-buprenorphine varied from 5 to 12. Slightly reduced gastrointestinal sounds were detected on abdominal auscultation of one horse 6 hours after buprenorphine administration though gastrointestinal sounds were subjectively assessed to be within normal limits at all other time-points in this horse and at all time-points in the other five horses. No signs suggestive of abdominal pain were detected at any point during the study.

Pharmacokinetic parameters
Plasma buprenorphine concentrations were detectable up to 480 minutes after buprenorphine administration in all horses and to 720 minutes after administration in two out of six horses. The plasma concentration declined in a curvilinear manner. The AUCs to the last sampling point and to infinity were 484.3 ± SD 88.2 ng mL⁻¹ minute⁻¹ and 521.3 ± 88.1 ng mL⁻¹ minute⁻¹, respectively. The extrapolated area after the last sampling point contributed 7.5 ± 1.6% of the total area to infinity. The pharmacokinetic parameters for IV buprenorphine for each horse are shown in Table 2. Plasma norbuprenorphine concentrations were below the limit of quantification of the assay (0.5 ng mL⁻¹) at all time-points in all horses.

Concentration–effect data
The relationship between the time after dosing, plasma drug concentration and peak pharmacodynamic anti-nociceptive effects are shown in Table 3. Data for thermal stimuli show a range of peak values as the cut-out temperature of 53 °C was breached in all six horses. As a result, the Tmax and Cmax values are shown as range and medians. More concise endpoints were recorded for the mechanical stimulus.

The PK modelling output, hysteresis plots and PK/PD fitting for both endpoints are plotted in Fig. 3 for a representative individual. PK/PD modelling was successful in all horses except one for which control data for mechanical threshold were inconsistent and unusable. Pharmacodynamic parameters and indicators of goodness of fit are presented in Table 4. The IC₅₀ for mechanical threshold was 6.8 ng mL⁻¹ (CV

Table 1 Median (range) step counts and step counts hour⁻¹ following administration of glucose and buprenorphine (10 µg kg⁻¹) IV to six horses. Period 1 was the time from treatment administration until 1 hour post-treatment, period 2 was 1–8 hours post-treatment and period 3 was eight to 24 hours post-treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Steps period 1 (steps hour⁻¹)</th>
<th>Steps period 2 (steps hour⁻¹)</th>
<th>Steps period 3 (steps hour⁻¹)</th>
<th>Steps total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose total steps</td>
<td>757 (171–982)</td>
<td>2154 (957–7811)</td>
<td>1684 (366–4037)</td>
<td>5270 (3502–10,436)</td>
</tr>
<tr>
<td>Glucose steps hour⁻¹</td>
<td>757 (171–982)</td>
<td>359 (156–1301)</td>
<td>112 (24–269)</td>
<td></td>
</tr>
<tr>
<td>Buprenorphine total steps</td>
<td>1516 (755–2329)</td>
<td>9882 (4939–20,449)</td>
<td>4342 (1624–10,676)</td>
<td>13,835 (9821–30,027)</td>
</tr>
<tr>
<td>Buprenorphine steps hour⁻¹</td>
<td>1516 (755–2329)</td>
<td>1647 (823–3408)</td>
<td>289 (108–711)</td>
<td></td>
</tr>
</tbody>
</table>
geometric mean 10.6%) with a sharp concentration-effect relationship \((n = 30.8)\). The IC\(_{50}\) for step count was 0.9 ng mL\(^{-1}\) with a more shallow concentration-effect relationship \((n = 1.3)\). The initial slope of the increase in response was 14.4 Newtons hour\(^{-1}\) and 2670 steps hour\(^{-2}\) for mechanical threshold and step count, respectively. PK/PD simulations with different doses are presented in Fig. 4. Doses higher than 10 \(\mu\)g kg\(^{-1}\) further increased locomotion but did not seem to increase mechanical threshold despite increasing the duration of analgesia.

**Discussion**

The pharmacokinetics of IV buprenorphine have been described in a number of domestic animal species including horses (Messenger et al. 2011; Davis et al. 2012) cats (Robertson et al. 2005), dogs (Abbo et al. 2008), rabbits, rats, sheep (Nolan et al. 1987) and man (Bullingham et al. 1980). A variety of assay techniques have been used in the different studies [radio-immunoassay (RIA); gas chromatography with mass spectrometry (GC-MS); liquid chromatography mass spectrometry (LC-MS)] each with its own level of sensitivity and specificity, as well as different dosing strategies and sampling regimens. The kinetics in sheep, cats and man were measured using an Iodine\(^{125}\)-labelled radio-immunoassay. This technique has the disadvantage that any cross-reactivity with buprenorphine-3-glucuronide or other buprenorphine metabolites could lead to errors in the measurement of plasma buprenorphine concentrations although the mechanisms of metabolism of buprenorphine in the animal species have not been fully established. In contrast, Abbo and colleagues used liquid chromatography-electrospray ionization tandem mass spectrometry for measurement of buprenorphine and three of its metabolites (norbuprenorphine, buprenorphine-3-glucuronide

### Table 2

Pharmacokinetic parameters calculated from concentration – time data for six horses after administration of buprenorphine (10 \(\mu\)g kg\(^{-1}\) IV)

<table>
<thead>
<tr>
<th>Horse</th>
<th>Weight (kg)</th>
<th>Lambda z (minutes(^{-1}))</th>
<th>MRT (minutes)</th>
<th>Vdss kg(^{-1}) (L kg(^{-1}))</th>
<th>Cl kg(^{-1}) (mL kg(^{-1}) minute(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>515</td>
<td>0.00409</td>
<td>164.9</td>
<td>2.46</td>
<td>14.91</td>
</tr>
<tr>
<td>B</td>
<td>487</td>
<td>0.00435</td>
<td>144.8</td>
<td>3.30</td>
<td>22.82</td>
</tr>
<tr>
<td>C</td>
<td>500</td>
<td>0.00462</td>
<td>150.0</td>
<td>3.13</td>
<td>20.96</td>
</tr>
<tr>
<td>D</td>
<td>490</td>
<td>0.00471</td>
<td>139.4</td>
<td>2.60</td>
<td>18.69</td>
</tr>
<tr>
<td>E</td>
<td>480</td>
<td>0.00499</td>
<td>116.5</td>
<td>2.62</td>
<td>22.45</td>
</tr>
<tr>
<td>F</td>
<td>508</td>
<td>0.00367</td>
<td>148.4</td>
<td>2.64</td>
<td>17.82</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>497 ± 13</td>
<td>0.00441 ± 0.00047</td>
<td>144 ± 15.9</td>
<td>2.79 ± 0.34</td>
<td>19.61 ± 3.04</td>
</tr>
</tbody>
</table>

### Table 3

Concentration [the concentration at which a maximal effect was observed (\(C\) at max effect, ng mL\(^{-1}\))], time (\(T_{\text{max}}\) minutes) and effect data for the six horses following 10 \(\mu\)g kg\(^{-1}\) IV buprenorphine. Where there were several time-points at which the cut-out temperature for the thermal threshold was reached in individual horses, the range of times and plasma concentration (together with extrapolated medians) are shown

<table>
<thead>
<tr>
<th>Horse</th>
<th>Thermal stimulus</th>
<th>Mechanical stimulus</th>
<th>Steps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(T_{\text{max}})</td>
<td>(C) at max effect</td>
<td>(T_{\text{max}})</td>
</tr>
<tr>
<td>A (median)</td>
<td>15–480 (90)</td>
<td>5.95–0.23 (1.43)</td>
<td>45</td>
</tr>
<tr>
<td>B (median)</td>
<td>15–360 (60)</td>
<td>4.09–0.27 (1.04)</td>
<td>240</td>
</tr>
<tr>
<td>C (median)</td>
<td>15–480 (90)</td>
<td>3.49–0.18 (1.06)</td>
<td>60</td>
</tr>
<tr>
<td>D (median)</td>
<td>15–480 (90)</td>
<td>3.25–0.18 (1.13)</td>
<td>60</td>
</tr>
<tr>
<td>E (median)</td>
<td>15–360 (60)</td>
<td>3.90–0.23 (1.61)</td>
<td>120</td>
</tr>
<tr>
<td>F (median)</td>
<td>15–480 (90)</td>
<td>3.98–0.21 (1.61)</td>
<td>45</td>
</tr>
</tbody>
</table>
and norbuprenorphine-3-glucuronide) in dog plasma (Abbo et al. 2008). Their results suggested that buprenorphine was less extensively metabolised in dogs compared to humans but the relationship between plasma concentration and antinociceptive or analgesic effect was not investigated.

Table 4 Pharmacodynamic parameters describing the effect of buprenorphine (10 μg kg⁻¹, IV) on mechanical threshold (Newton) and locomotion (step count hour⁻¹) in six horses: Parameters estimates were obtained using indirect PK/PD models including control data fitted simultaneously. Step count are presented as geometric mean ± coefficient of variation% (n = 6) and mechanical threshold data are presented as mean ± SD (n = 5). Iₘₐₓ, maximum inhibitory effect (limited by cut out of mechanical threshold testing at 15 N); IC₅₀, plasma concentration to obtain 50% of the effect; Kᵢᵣ₀, zero-order rate constant for build-up of the response; R₀, common baseline fitted for buprenorphine and control functions; n, slope of the concentration-effect relationship. Goodness of fit for both models relied on visual assessment of residuals and AIC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Kᵢᵣ₀</th>
<th>IC₅₀</th>
<th>Iₘₐₓ</th>
<th>R₀</th>
<th>n</th>
<th>Goodness of fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Newton hour⁻¹ or steps)</td>
<td>(ng mL⁻¹)</td>
<td>(no unit)</td>
<td>(no unit)</td>
<td>(no unit)</td>
<td>AIC</td>
<td>Coefficient correlation (r)</td>
</tr>
<tr>
<td>Mechanical threshold (Newton)</td>
<td>17.4 ± 508%</td>
<td>6.84 ± 36.7%</td>
<td>0.53 ± 10.6%</td>
<td>3.98 ± 14.6%</td>
<td>30.8 ± 156%</td>
<td>106 ± 23.1</td>
</tr>
<tr>
<td>Step count (step hour⁻¹) mean ± SD</td>
<td>2670 ± 746</td>
<td>0.90 ± 0.47</td>
<td>Fixed to 1</td>
<td>56.3 ± 21.5</td>
<td>1.3 ± 0.5</td>
<td>617 ± 18.7</td>
</tr>
</tbody>
</table>
This present study measured the kinetics of buprenorphine at a dose of 10 $\mu$g kg$^{-1}$ given IV to the horse. Data were collected by a team of three experienced investigators in order to ensure precision in the time of blood sample collection relative to the time of drug administration. Use of a LC-tandem MS assay enabled detection of drug concentrations, above the limit of detection, to 480 minutes in all horses. The estimates produced for Clp and Vdss by the non-compartmental approach are comparable to values determined by compartmental methods. In this study, venous rather than arterial sampling was used so making the delineation of the fast phase (distribution) half-life liable to inaccuracies. Furthermore the sampling regimen used in this study (1, 2, 4, 6 and 10 minutes) would tend to under-calculate its value.

The estimates for Vdss in the horse are in keeping with those for man (Bullingham et al. 1980) and sheep (Nolan et al. 1987), and in broad agreement with previously reported values in horses (Messenger et al. 2011; Davis et al. 2012) as well as the values predicted by the allometric analyses of Sear et al. (2007).

Although we report higher clearance for IV buprenorphine compared with that reported by Messenger et al. (2011) and Davis et al. (2012), this may reflect differences in sampling strategy, dose given, or simply inter-individual variation within small study populations. Other studies have shown increased liver blood flow secondary to the cardiotimulatory effects of buprenorphine after doses of 10 $\mu$g kg$^{-1}$ (Carregaro et al. 2006). There was some evidence that buprenorphine increased heart rate in the present study. In anaesthetized human subjects, there is a reduction in buprenorphine clearance secondary to a decrease in hepatic blood flow from 619 to 1220 mL minute$^{-1}$ (Bullingham et al. 1980; Hand et al. 1990; Amani et al. 1997). Although there is an apparent increase in systemic clearance, the apparent volumes of distribution are similar in our study and the previous studies in horses (Messenger et al. 2011; Davis et al. 2012). In a preliminary report, Sear et al. (2007) found a relationship between body weight and both systemic clearance and apparent volume of distribution at steady state using an allometric power equation based on data over a range of species from the rat to man (Sear et al. 2007). Inclusion of data for the horse did not influence this relationship. This suggests that the kinetics of buprenorphine on a mg per kg bodyweight basis may be assumed for a wide range of species, so aiding dosage recommendations.

There are fewer data on the metabolism of buprenorphine in animal species. Bartlett et al. (1980) reported a similar metabolic profile for buprenorphine in the dog and humans with very limited production of buprenorphine metabolites following parenteral administration. However in dogs, Garrett & Chandran (1990) found high concentrations of the metabolite buprenorphine-3-glucuronide (B3G) within 2 minutes of IV dosing, with negligible formation of the metabolite norbuprenorphine-glucuronide (NBG). A more recent study in dogs investigating the pharmacokinetics of buprenorphine after IV administration of clinical doses did not report plasma concentrations of buprenorphine metabolites (Andaluz et al. 2009). The metabolites of buprenorphine in cats have also not been identified (Robertson et al. 2005), although because cats have poor glucuronide capacity (Court & Greenblatt 1997a,b) generation of B3G is unlikely. Studies in man are limited. Hand et al. (1990) found no detectable metabolites after single IV dosing in both healthy and renally-impaired anaesthetized patients. However, when the drug was given by continuous infusion in the intensive care unit, both B3G and NBG were detected; and were present in
greater concentrations in patients with reduced renal function. The concentrations of B3G were 2–3 times those of NBG. In the present study in horses, plasma norbuprenorphine concentrations were below the limit of quantitation in every sample. This may indicate either that the horse does not produce this metabolite, or that it is rapidly cleared from the blood.

In the present study, all horses received buprenorphine and the study was not conducted as a cross over design such that contemporaneous pharmacodynamic data from the same horses without buprenorphine treatment could be compared. This was justified because similar pharmacodynamic data had been collected previously in an identical manner, from the same horses following administration of glucose 5%. These control data are reported in a separate publication (Love et al. 2012). It was therefore deemed ethically unacceptable to repeat the measurements of thermal and mechanical nociceptive thresholds in the same horses following glucose 5% (Slingsby 2010). The control data (Love et al. 2012) indicate that treatment with glucose 5% did not produce thermal or mechanical antinociception, nor was there any additional effect of time on threshold measurements. Occasional and inconsistent increases in threshold temperatures were seen in individuals, but were associated with the horses appearing to be distracted following handling and drug administration. Control data also demonstrated that treatment with glucose 5% did not cause a significant change in heart rate or respiratory rate compared to baseline over a similar 24 hour period after administration. In the present study, changes in pharmacodynamic variables over time that differed to variables reported for the same horses treated with glucose 5% were therefore attributed to administration of buprenorphine.

In a separate study the dose dependent effects of buprenorphine on thermal and mechanical thresholds were previously reported, and buprenorphine 10 µg kg⁻¹ IV caused similar changes in nociceptive thresholds to those reported here (Love et al. 2012). In the present study, all horses skin temperature increased above the baseline measurements from 15 minutes after buprenorphine administration although rectal temperatures remained within normal limits. This increase in skin temperature could have been caused by a number of factors: a direct effect of buprenorphine, an effect of the testing apparatus covering the skin and reducing heat loss, high environmental temperatures and/or secondary to locomotor stimulation. Carregaro et al. (2006) reported increased locomotor and sympathetic nervous system activity in pain free horses administered 10 µg kg⁻¹ buprenorphine IV, attributed to central nervous system stimulation, that was also associated with an increase in rectal temperature. In the present study rectal temperature was unchanged despite the increase in motor activity, therefore it is difficult to attribute the increase in skin temperature solely to increased motor activity as a concurrent increase in rectal temperature would be predicted. An increase in core body temperature has been reported following administration of buprenorphine in experimental horses (Ilback et al. 2008) and the authors suggested that this may be due to a possible effect of buprenorphine on thermoregulatory function although no work has been published, to date, to confirm or refute this hypothesis. In studies in cats where a similar thermal threshold testing system has been used to measure skin and threshold temperatures buprenorphine administration has not influenced skin temperature (Robertson et al. 2003, 2005; Steagall et al. 2006, 2007, 2009b) although an increase in skin temperature was detected following hydromorphone (Wegner et al. 2004). In a clinical population of cats, hydromorphone administration was commonly associated with post-operative hyperthermia (rectal temperature >40 °C) while although hyperthermia did occur in a few cats that received buprenorphine this was much less common (Niedfeldt & Robertson 2006). The relatively high environmental temperatures and sun exposure on the days on which this study were performed may have influenced skin temperatures. A direct effect of sun exposure may account for the elevated skin temperatures in the face of normal rectal temperatures. A very recent study, published after this study was carried out, investigated the effects of ambient temperature on thermal thresholds in horses that were measured using a contact thermode placed on the nostrils or withers (Poller et al. 2013). Poller et al. (2013) compared thermal thresholds measured during ambient temperatures that were either <10 °C or >20 °C and found thresholds to be lower at warmer ambient temperatures although the mechanism is unclear. Mean ambient temperature was >20 °C at all times during the study, although there are currently insufficient published data to predict the effect of small changes in ambient temperature (e.g. 2–3 °C) on thermal threshold in horses. In any case, because much of the data for thermal threshold following buprenor-
Buprenorphine administration were censored at 53 °C, any effect of variability in ambient temperature on threshold measurements will have been limited. Furthermore, despite the potential confounding effect of environmental temperature on thermal thresholds, the duration of the antinociceptive effect to the thermal stimulus was similar to that reported in a previous study conducted during the winter months using the same horses (Love et al. 2012).

The inclusion of a ‘cut-out’ temperature (53 °C) in the thermal threshold testing equipment was essential to prevent damage to the horses’ skin. Since the ‘cut out’ value was reached from 15 minutes until between 8 and 12 hours after buprenorphine administration, the scope to relate the thermal antinociceptive effects to the pharmacokinetics is limited and it was not possible to detect the timing of the maximal effect. In this study a more rapid onset of effect was detected than that reported in sheep (Nolan et al. 1987) and cats (Steagall et al. 2007).

The use of a mechanical threshold stimulus and locomotor activity, as measured by step counting, has allowed a more complete PK/PD relationship to be delineated for the horse. There are few data on the dynamic profiles of IV buprenorphine. The studies in sheep, cats and the rat show a delay between the peak plasma buprenorphine concentration after IV dosing and the peak antinociceptive effect (Nolan et al. 1987; Robertson et al. 2005). In the present study, the first time point of pharmacodynamic data collection was 15 minutes after buprenorphine administration, when thermal nociceptive threshold was maximum. This suggests that there is at most only a very short blood-brain equilibration delay between buprenorphine administration and antinociceptive effect in equidae.

When administered to the horse, buprenorphine caused typical opioid-mediated effects. In common with previous studies administering buprenorphine, without a concurrent sedative drug, to pain free horses (Carregaro et al. 2007; Messenger et al. 2011; Davis et al. 2012), locomotor activity was increased compared with activity recorded over a similar time period in horses administered 5% glucose. However, in contrast to the previous studies in the present study, behavioural signs of excitation were not documented. Individual variability in behavioural responses to buprenorphine has been previously reported (Messenger et al. 2011). Locomotor stimulation is commonly observed after the administration of butorphanol (Nolan et al. 1994) and other opioids to healthy, pain-free horses (Pascoe & Taylor 2003) but is less frequently observed when opioids are administered to horses experiencing pain (Mircica et al. 2003). Locomotor stimulation may have been responsible for the increases in heart rate detected after buprenorphine administration. Despite the statistically significant increase in heart rates they remained within clinically acceptable limits throughout the study. Other opioid effects in horses include a reduction in gastrointestinal motility, which has been previously reported in horses administered buprenorphine (Carregaro et al. 2006; Messenger et al. 2011; Davis et al. 2012). Following a single dose of buprenorphine, minimal effects on gastrointestinal motility were detected and no horses developed abdominal discomfort.

The present study indicates the likely suitability of buprenorphine for equine perioperative analgesia; this has now been demonstrated in ponies where it was used for post-castration pain relief (Love et al. 2013).

Acknowledgements

Alstoe Animal Health provided financial support for this study.

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Received 28 March 2013; accepted 8 October 2013.