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Acepromazine pharmacokinetics: A forensic perspective ☆

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ABSTRACT

Acepromazine (ACP) is a useful therapeutic drug, but is a prohibited substance in competition horses. The illicit use of ACP is difficult to detect due to its rapid metabolism, so this study investigated the ACP metabolite 2-(1-hydroxyethyl)promazine sulphoxide (HEPS) as a potential forensic marker. Acepromazine maleate, equivalent to 30 mg of ACP, was given IV to 12 racing-bred geldings. Blood and urine were collected for 7 days post-administration and analysed for ACP and HEPS by liquid chromatography–mass spectrometry (LC–MS).

Acepromazine was quantifiable in plasma for up to 3 h with little reaching the urine unmodified. Similar to previous studies, there was wide variation in the distribution and metabolism of ACP. The metabolite HEPS was quantifiable for up to 24 h in plasma and 144 h in urine. The metabolism of ACP to HEPS was fast and erratic, so the early phase of the HEPS emergence could not be modelled directly, but was assumed to be similar to the rate of disappearance of ACP. However, the relationship between peak plasma HEPS and the *y*-intercept of the kinetic model was strong ($P = 0.001$, $r^2 = 0.72$), allowing accurate determination of the formation pharmacokinetics of HEPS. Due to its rapid metabolism, testing of forensic samples for the parent drug is redundant with IV administration. The relatively long half-life of HEPS and its stable behaviour beyond the initial phase make it a valuable indicator of ACP use, and by determining the urine-to-plasma concentration ratios for HEPS, the approximate dose of ACP administration may be estimated.

Introduction

Acepromazine (ACP) is a commonly used phenothiazine tranquilizer administered to reduce excitement and stress during various veterinary procedures, and as a pre-anaesthetic agent in barbiturate anaesthesia (Posner and Burns, 2009). Additionally, it is administered to nervous and/or difficult horses during training, transport and confinement to relieve anxiety. Its tranquilizing effects are seen without appreciably affecting co-ordination therefore allowing better control of excitable horses (Posner and Burns, 2009). The usual route of administration is either IV or IM, although an oral gel formulation is also available. In most racing and equestrian jurisdictions, ACP is classed as a prohibited substance (ARB, 2011; ARCI, 2011; FEI, 2011). The definition of a prohibited substance is quite extensive and varies between jurisdictions, but generally, it is the term applied to a group of substances declared by a jurisdiction as not being allowed in the horse's system at the time of competition (ARB, 2011).

The illicit use of ACP is difficult to detect because the drug is metabolised rapidly and extensively. Studies on the pharmacokinetics of ACP confirm this, but as most investigations have focussed on its clinical use, there is far more information about the parent drug than its metabolites (Ballard et al., 1982; Hashem and Keller, 1993; Marroum et al., 1994; Chou et al., 1998, 2002). These previous studies report a β-phase half-life of elimination for ACP ranging from 50 to 184.8 min (Ballard et al., 1982; Hashem and Keller, 1993; Marroum et al., 1994) and a mean retention time (MRT) of 1.43 h (Marroum et al., 1994) to 1.54 h (Hashem and Keller, 1993). Chou et al. (1998, 2002) reported the detection of ACP in plasma up to 48 h post IM administration. Clearance rates of between 2.5 and 3 L/kg/h have also been reported (Ballard et al., 1982; Hashem and Keller, 1993; Marroum et al., 1994).

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The presence of ACP in blood and urine can vary significantly depending on administration of a single or multiple doses and the exercise status of the horse (Chou et al., 2002). These previous pharmacokinetic studies were done in only four to six horses and showed a large variation between individual horses (Ballard et al., 1982; Hashem and Keller, 1993; Marroum et al., 1994). As stated by Ballard et al. (1982), although ACP is prohibited by racing authorities and other competitive horse organisations, it does have frequent legitimate uses. Therefore it is essential that reliable pharmacokinetic data are available for both competitors and competition organisers alike.

Weir and Sanford (1972) identified an unconjugated sulphoxide compound in urine as the major metabolite of ACP. Consistent with this finding, Dewey et al. (1981) described three compounds in horse urine derived from the metabolism of ACP, two of which were determined only after hydrolysis, and a third unconjugated metabolite, which was categorised as 2-(1-hydroxyethyl)promazine sulphoxide (HEPS). There has been little subsequent investigation of HEPS, and to the authors' knowledge, no reported pharmacokinetic studies.

The aim of this study was to produce reliable pharmacokinetic data and excretion times for ACP in both plasma and urine. Further, the utility of HEPS as a forensic marker for illicit ACP administration was investigated by producing an accurate model of HEPS pharmacokinetics in both plasma and urine. From this it could be determined if HEPS is a viable marker for the detection of ACP as a prohibited substance.

Materials and methods

Horses and drug administration

Twelve unfit mature geldings (8.5 ± 0.8 years old) of Thoroughbred (4/12) or Standardbred (8/12) breeding were used in this study. The horses had a mean bodyweight (BW ± SEM) of 551.3 ± 14.3 kg and a body condition score of 3, on a scale of 0–5 (Carroll and Huntington, 1988). Lucerne hay was fed at 2% BW/day as maintenance. Horses were housed in individual 3 m × 3 m stalls for 7 days from the day before the study. They were exercised at the walk for 1 h each day for welfare reasons on a mechanical horsewalker (Irongate), including on the afternoon of the drug administration day after the 6 h sampling.

On the day of drug administration, catheters (14 G, Angiocath, Becton Dickinson) were inserted into the jugular vein of each horse after the administration of a local anaesthetic (0.5–1.0 mL, Ilium Lignocaine 20). Blood and urine samples were collected and then all horses were administered 3 mL (~30 mg) of ACP 10 (Delvet) (acepromazine maleate (ACP maleate) 13.5 mg; equivalent to ACP 10 mg/mL) by IV injection at 08:00 h. Blood samples were taken at 5, 10, 20 and 40 min, and at 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 12 h after injection. Blood samples were collected into 10 mL lithium heparin Vacutainer (Terumo) and centrifuged at 4 °C (10 min; 2800 *g*), before the plasma was harvested and stored at −20 °C until analysed. Urine samples were collected after 2, 4, 6, 8 and 12 h after drug administration, plus a 48 h total urine collection was performed. On the day following drug administration, additional blood and urine samples were collected at 24 h and 32 h, with further samples collected every 24 h up to 144 h after drug administration.

All procedures were performed with the approval of the Animal Care and Ethics Committee of Charles Sturt University, and were in accordance with the Australian Code of Practice for the Care and use of Experimental Animals for Scientific Purposes (NHMRC, 2004).

Sample analysis

Both plasma and urine samples were analysed for ACP and HEPS using LC/MS. This equipment is routinely used by racing laboratories worldwide and as this study is primarily aimed at the detection of prohibited substances, it is appropriate to use this technology above other methods used previously (Dewey et al., 1981; Chou et al., 1998).

Chemicals

Acepromazine maleate was purchased from the National Measurement Institute. Propionylpromazine hydrochloride was purchased from Sigma. 2-(1-Hydroxyethyl)promazine sulfoxide maleate (HEPS maleate; 1 mg/mL solution in methanol) and 2-([1,2,2,2-$^2H_4$]-1-hydroxyethyl)promazine sulfoxide maleate (HEPS-d_4 maleate; 1 mg/mL solution in methanol) were purchased from Frontier BioPharm. IST Isolute HCX solid phase extraction cartridges (3 mL; 130 mg) were purchased from Biotage.

Sample preparation: plasma

Aliquots of plasma (0.5 mL) were spiked with propionylpromazine hydrochloride and HEPS-d_4 maleate equivalent in each case to 27 ng/mL free base, then were diluted with methanol (0.8 mL) and acetic acid (0.1 M; 1.7 mL) and centrifuged (5 min; 1500 *g*) to precipitate proteinaceous material. The supernatant fractions were decanted and loaded onto IST Isolute HCX solid phase extraction cartridges which had previously been conditioned with methanol (3 mL) and acetic acid (0.1 M; 3 mL). The cartridges were washed with acetic acid (0.1 M; 3 mL) and methanol (3 mL), then were dried briefly and eluted with ethyl acetate:methanol:ammonium hydroxide (92:5:3 v/v/v; 3 mL). The eluates were evaporated to dryness at 60 °C under a stream of nitrogen before being reconstituted in isopropanol (10 μL) and ammonium acetate (20 mM; 40 μL) for LC–MS analysis.

Sample preparation: urine

Aliquots of urine (1 mL) were spiked with propionylpromazine hydrochloride equivalent to 8 ng/mL free base and HEPS-d_4 maleate equivalent to 400 ng/mL free base, then were diluted with acetic acid (0.1 M; 2 mL) and centrifuged (5 min; 1500 *g*) to precipitate particulate material. The supernatant fractions were decanted, then were extracted and reconstituted for LC–MS analysis as described above for the plasma samples.

LC–MS analysis

Samples were analysed using a Waters Acquity UPLC system equipped with a Waters Acquity UPLC BEH C18 analytical column (2.1 × 50 mm, 1.7 μm) and Phenomenex Security-Guard C18 guard column (4 × 2 mm) interfaced to an Applied Biosystems 4000 Q-Trap mass spectrometer. Sample injections (10 μL) were made into an initial mobile phase comprising 100% aqueous ammonium acetate (20 mM; solvent A). After 0.2 min, acetonitrile (solvent B) was introduced and increased in a linear gradient over 2.3 min to a final proportion of 60%. This constitution was held for a further 1.5 min, then was returned to the starting conditions and equilibrated for 2 min prior to the next injection. The flow rate was maintained at 400 μL/min and the column temperature at a constant 50 °C throughout. The MS was operated in positive ion atmospheric pressure chemical ionisation (APCI) mode with ion source conditions optimised for ACP. Data were collected in multiple reaction monitoring (MRM) mode in two segments: segment one monitored HEPS and HEPS-d_4, while segment two monitored ACP and propionylpromazine. MS parameters for segment one were optimised for HEPS and for segment two, ACP with 4–5 transitions were used to monitor each analyte. The chromatograms for each analyte were summed for quantitative purposes.

Quantitation: plasma

Calibration standards were prepared by spiking pooled blank equine plasma with ACP maleate and HEPS maleate equivalent in each case to 0, 1, 3, 9, 27 and 81 ng/mL free base. Quality control samples were prepared by spiking pooled blank equine plasma with ACP maleate and HEPS maleate equivalent in each case to 4 ng/mL free base (low control) and 50 ng/mL free base (high control). The spiking solutions for the calibration standards and quality control samples were prepared from separately weighings (ACP) or dilutions (HEPS). All samples were analysed in duplicate.

Quantitation: urine

Calibration standards were prepared by spiking pooled blank equine urine with ACP maleate equivalent to 0, 1, 2, 4, 8 and 16 ng/mL free base and HEPS maleate equivalent to 0, 1, 8, 64, 512 and 4096 ng/mL free base. Quality control samples were prepared by spiking pooled blank equine urine with ACP maleate equivalent to 4 ng/mL free base (low control) and 10 ng/mL free base (high control) and HEPS maleate equivalent to 8 ng/mL free base (low control) and 2500 ng/mL free base (high control). The spiking solutions for the calibration standards and quality control samples were prepared from separate weighings (ACP) or dilutions (HEPS). All samples were analysed in duplicate.

Table 1
Accuracy and precision data of the LC/MS analysis of ACP and HEPS in plasma and urine, using low and high control standards appropriate to concentrations expected from biological samples.

Analyte/matrix	Concentration (ng/mL)	Accuracy (% of nominal)	Precision (%CV)
ACP/plasma	4	102–109	3.6
ACP/plasma	50	95–103	5.3
ACP/urine	4	89–104	7.7
ACP/urine	10	95–104	4.4
HEPS/plasma	4	88–98	7.9
HEPS/plasma	50	95–104	6.4
HEPS/urine	8	112–119	3.1
HEPS/urine	2500	103–111	4.5