IDENTIFICATION AND DETECTION OF THE MAJOR EQUINE URINARY METABOLITE OF REMIFENTANIL

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ABSTRACT

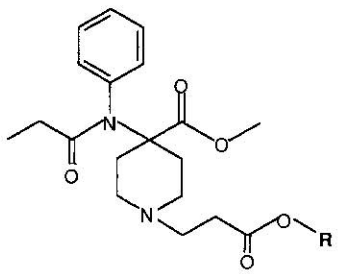
Remifentanil (4-methoxycarbonyl-4-[(1-oxopropyl) phenylamino]-1-piperidinepropionic acid methyl ester) is a μ-opioid receptor agonist and an ARCI Class 1 drug with considerable abuse potential in racehorses. The drug's major equine urinary metabolite is 4-methoxycarbonyl-4-[(1-oxopropyl) phenylamino]-1-piperidinepropionic acid, an ester hydrolysis product of remifentanil.

Remifentanil HCl administration at 5 mg iv evidenced locomotor responses which established the clinical efficacy of this low dose. Post administration urine samples were analysed by a fentanyl-group ELISA assay (Neogen Corp., Kentucky, USA), which readily detected fentanyl equivalents in these samples. Solid phase extraction and trimethylsilyl (TMS) derivatisation of extracts was followed by GC/MS analysis, which showed that the urine samples contained parent remifentanil in low concentrations with a peak at 1 h. A major peak was identified as representing 4-methoxycarbonyl-4-[(1-oxopropyl)phenylamino]-1-piperidinepropionic acid, arising from ester hydrolysis of remifentanil. This metabolite reached its maximal urinary concentrations at 1 h and was present at up to 10-fold greater concentrations than parent remifentanil. Base hydrolysis of remifentanil yielded a carboxylic acid with the same mass spectral characteristics as those of the equine metabolite.

These data indicate therefore that remifentanil administration results in readily detectable amounts of 4-methoxycarbonyl-4-[(1-oxopropyl)phenylamino]-1-piperidinepropionic acid in equine urine. We recommend that screening and confirmation tests for this ARCI Class I drug be optimised for this metabolite to ensure forensic control of remifentanil.

INTRODUCTION

Remifentanil is the methyl ester of 4-methoxycarbonyl-4-[(1-oxopropyl)phenylaminol-1-piperidinepropionic acid (Fig 1, Burkle *et al.* 1996). It has properties analogous to those of structurally related phenylpiperidines such as fentanyl and sufentanil, mediated by interaction with μ-opioid receptor (Michelsen *et al.* 1996). Physiological changes in man consistent with potent μ-receptor



Compound	R	MW
Remifentanil	CH3	376
1-piperidine propionic acid metabolite	H	362
1-piperidine propionic acid metabolite TMS ester	TMS	434

Fig 1: Structure of remifentanil and its proposed equine 1-piperidinepropionic acid metabolite. Inset table shows the structure of the R-group in each case, where TMS represents trimethylsilyl and occurs after derivatisation of the 1-piperidinepropionic acid with BSTFA + 1% TMCS to a 1-piperidinepropionic acid TMS ester for GC/MS chromatographic visualisation.

agonists include analgesia and sedation. Adverse effects include ventilatory depression, nausea, vomiting, muscular rigidity and bradycardia. Remifentanil has fewer adverse homodynamic effects than morphine due a lack of histamine release upon injection (Egan 1995). Bradycardia associated with remifentanil can be effectively antagonised by naloxone (Amin *et al.* 1999).

Remifentanil is classified as an ultra-short-acting phenylpiperidine opioid analgesic owing to the susceptibility of the methyl ester on the propionic acid side-chain to hydrolytic cleavage by blood and tissue non-specific esterases (Cox *et al.* 1999). The main remifentanil metabolite in man is the carboxylic acid hydrolysis product [Glaxo Designation # GR90291] (Hoke *et al.* 1997). GR90291 has been identified in canine urine samples as a pure μ-agonist with a potency less than 1/2000 that of remifentanil (Michelsen *et al.* 1996).

μ-opioid receptor agonists produce different effects in the horse than in man; specifically they increase locomotor activity and stimulate movement at a pace somewhere between a brisk trot and a gallop (Kamerling 1999). As a locomotor stimulant and potent analgesic, remifentanil thus has the potential to increase performance in racehorses, leading to its classification by the Association of Racing Commissioners International as a Class 1 agent with the highest potential to affect the performance of a racing horse. In light of its significant potential for racetrack abuse, this research focused on generating methods for detection and confirmation of remifentanil and its metabolites in equine urine.

MATERIALS AND METHODS

Horses/drugs/clinical signs

Mature Thoroughbred mares weighing 422–503 kg were used throughout and maintained on grass hay and feed (12% protein), which was a 50:50 mixture of oats and an alfalfa-based protein pellet. They were fed twice daily, vaccinated annually for tetanus, and de-wormed quarterly with ivermectin. A clinical examination was performed before each experiment. During experimentation, the horses were provided water and hay *ad libitum* and the animals were managed according to the rules and regulations of the University of Kentucky Institutional Animal Care Use Committee, which also approved the experimental protocol.

Remifentanil (Ultiva, Glaxo Wellcome, Inc., North Carolina, USA) was administered iv in doses of 1 and 10 mg (2 x 5 mg doses given 10 min apart). Urine samples were collected at 0, 1, 2, 4, 6, 8, 24, 48 and 72 h after administration and stored at -20°C.

ELISA quantitation of remifentanil equivalents in urine

The one-step ELISA assay used for this analysis has been described by Weckman *et al.* (1988). Standard curves for remifentanil and alfentanil in the Fentanil Group ELISA test kit (Neogen Corporation, Kentucky, USA) were constructed using assay buffer. The assays were started by adding 20 μl of either the standard, test or control samples to each well of the kit. During the test, the presence of remifentanil equivalents in the sample competitively prevented the binding of the hapten-HRP (horse radish peroxidase) complex to the antibody. Since the HRP enzyme is responsible for the colour-producing reaction in the ELISA, the log of the concentration of remifentanil and its metabolites in the sample is inversely related to the percentage of maximal optical density of the test well, which was determined at a wavelength of 650 nm with an automated microplate reader (Bio-Tek Instruments, Vermont, USA) approximately 30 min after addition of substrate. All assay reactions were run at room temperature (22°C). Also, urine samples collected from a horse dosed with remifentanil (1 mg, iv) were screened with this test kit to determine the response of the ELISA kit to remifentanil and its metabolite(s).

Urine sample preparation

To facilitate passage of equine urine through SPE (solid phase extraction) columns, urine was first sonicated with a Fisher Scientific Sonic Dismembrator Type 60 equipped with an Ultrasonic Convertor horn type CML-3 (Fisher Scientific, Pennsylvania, USA). Three pulses 30 s in duration were sequentially applied with increasing intensity for each pulse (30, 60, and 90 watts). Samples were kept on ice throughout the procedure to minimise heating.

Urine extraction procedure

Clean-screen (#ZSDAU-020; Worldwide Monitoring, Pennsylvania, USA) SPE columns were conditioned by sequential addition of 2 ml methanol, 5 ml deionised water, 2 ml 100 mM sodium phosphate buffer (pH 6.0), after which 5 ml sonicated urine samples buffered with 2 ml 100 mM sodium phosphate buffer (pH 6.0) were loaded. Each column was then washed sequentially with 3 ml deionised water, 1 ml 0.1 M hydrochloric acid, and 3 ml methanol. The drug metabolites were eluted

with 2 ml dichloromethane/isopropanol/ NH_4OH, 78/20/2, v/v/v. Dimethylformamide (15 µl) was added as a keeper solvent. The eluent was evaporated to dryness at ~40°C under a stream of nitrogen gas. The sample was resuspended in 200 µl of dichloromethane, transferred to an autosampler vial equipped with a 200 µl spring-loaded insert, and again evaporated to dryness at ~40°C under a stream of nitrogen gas. For derivatisation, each dried sample was dissolved in 40 µl of N,O-bistrimethylsilyltrifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) (Pierce Chemicals, Illinois, USA), vortexed briefly, and incubated at 70°C for 30 min to produce the trimethylsilyl (TMS) derivative of remifentanil carboxylic acid metabolites.

GC/MS analysis

TMS derivatives (1 µl) were injected in splitless mode into the 250°C injector port of a Hewlett-Packard 6890/5972 GC/MSD (Georgia, USA), with sample separation on a 30 m long x 0.25 mm id x 0.25 µ film thickness DB-5MS 5%-phenyl-95%-methylpolysiloxane column (J&W Scientific, California, USA) with oven programming beginning at 70°C (held for 2 min), then ramping at 20°C/min to 280°C (held for 12 min). Helium flow rate was 1 ml/min. Under these conditions, remifentanil eluted at 13.6 min retention time, and the carboxylic acid metabolite-TMS derivative eluted at 14.1 min. The mass spectrometer was set to acquire from 50–550 m/z at 1.53 scans/s with a threshold of 150. In quantitative experiments, selected ion monitoring (SIM) was performed for ions 319, 303, 227, 212 and 168 m/z for remifentanil, and 377, 303, 285, 270 and 226 m/z for remifentanil carboxylic acid-TMS. Mass Spec Calculator Pro, Version 4.03 (Quadtech Associates, Inc., 1998) assisted in the interpretation of full scan mass spectra where necessary. Extracts were screened manually for metabolites by focusing on predicted molecular weights for various hydrolytic and dealkylated products and their glucuronide or sulphate conjugates.

Preparation of 4-methoxycarbonyl-4-[(1-oxopropyl)phenylamino]-1-piperidinepropionic acid

Remifentanil-HCl (20 mg) was dissolved in 1 ml methanol:water (1:1) and a 0.1 N sodium hydroxide solution was added dropwise until pH ~12 was achieved. The mixture was stirred at room temperature overnight, acidified by addition of 0.1 N hydrochloric acid to pH ~4, and evaporated under reduced pressure to dryness. Chloroform (5 ml) was added, the mixture was stirred for 1 h and filtered. Chloroform was evaporated to obtain a colorless oil (~10 mg).

MS/MS (general)

Full scan electrospray ionisation (ESI) mass spectra were obtained on analytical standards at 10 µg/ml in 50:50 acetonitrile:0.05% formic acid (aq), pH ~3, by infusion at 0.6 ml/h with a Harvard syringe pump (Massachusetts, USA) into the electrospray probe of a Quattro II MS/MS (Micromass, Beverly, MA) set in positive ion mode. Negative mode spectra were obtained similarly but with dissolution in 50:50 acetonitrile:0.5% (v/v) NH_4OH (aq, from conc.), pH ~10. Spectra were optimised by combining 1–2 min of uniformly acquired data, background subtraction and peak smoothing.

MS/MS tuning

The Quattro II MS was tuned for positive mode by direct infusion of 10 ng/µl remifentanil in 50:50 acetonitrile:0.05% formic acid (aq). The peak shape and intensity of the monoprotonated remifentanil 377 m/z ion were optimised by adjustment of capillary, HV lens, cone voltage, skimmer lens and RF lens settings. Negative ion mode was optimised similarly with focus placed on the monodeprotonated remifentanil carboxylic acid 361 m/z ion as visualised in 50:50 acetonitrile:0.5% (v/v) NH_4OH (aq, from concentrated). Collision gas (argon) and collision energy were adjusted for collisionally-induced dissociation (CID) in the central hexapole by optimising settings as needed for MS^2. Generally the collision gas was set to 1 x 10^{-3} mbar. In general, for positive mode the source cone voltage was set at +35 v, the collision energy was set at -25v, the capillary of the ESI probe was set at +3.5 kvolts, skimmer was set at 1.0 v and the HV lens was set at +0.7 kvolts. For negative mode the source cone voltage was set at 25v, the collision energy was set at -24v, the capillary of the ESI probe was set at +2.9 kvolts, skimmer was set at 1.9v and the HV lens was set at +0.65 kvolts. Source temperature was set at 100°C.

Direct examination of urine for glucuronide or sulphate metabolites by MS/MS

Urine from horses taken 0, 2, 4, 6 or 8 h after treatment with 1 mg of remifentanil was passed through a ~3,000 m.w. cutoff Centrifree filter (Amicon, Inc., Massachusetts, USA) to remove high molecular weight materials. Specifically, 900 µl of urine was centrifuged for 90 min at 1,200 *g* in a swinging bucket rotor (Type AH-4) in an

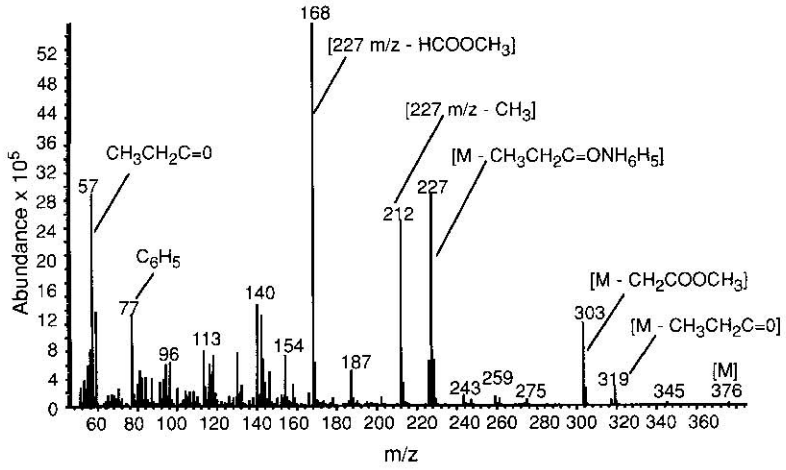


Fig 2: Electron impact mass spectrum of remifentanil peak seen at 13.6 min by gas chromatography, with proposed assignment of structures to major m/z values.

AccuSpinFR centrifuge (Beckman, California, USA). The filtrate was diluted 1:10 with 50:50 acetonitrile:0.5% (v/v) NH_4OH (aq, from concentrated) for negative mode. The mixture was infused at 0.9 ml/h via a Harvard syringe pump equipped with a 500 µl Hamilton gas-tight syringe. Infusion was direct into the electrospray probe of the Quattro II MS/MS for electrospray ionisation (ESI) mass spectrometry with the source tuned as described above.

RESULTS

Clinical responses

Remifentanil (1 mg iv) caused no discernible effect on locomotor or behavioural activity. In a subsequent experiment, remifentanil was administered in 2 x 5 mg doses. Within 1 min of the first 5 mg dose, the horse paced rapidly around the stall, an activity which lasted about 5 min. When pacing ceased, a second 5 mg injection was administered and the locomotor effects were similar in intensity and duration to those seen following the first injection. Mild sweating was observed about 5 min after the second injection, and it persisted for about 10 min.

TABLE 1: Remifentanil concentrations in urine after intravenous administration of 1 mg of remifentanil as determined by GC/MS

Time post administration of remifentanil (h)	Remifentanil concentration (ng/ml)
0	0
1	800
2	260
4	175
6	50
8	4
24	0

ELISA detection

Use of Neogen's Fentanil Group ELISA test kit demonstrated that the standard curves for remifentanil and alfentanil were equivalent (I_{50} ~0.4 ng/ml), showing that the test kit readily detects remifentanil (data not shown). The ELISA kit was applied to urine samples from a horse treated with iv remifentanil at a dose of 1 mg, a sub-clinical dose in terms of observable clinical response. The inhibitory effect of remifentanil equivalents in the urine samples was maximal at 1 h after dosing and thereafter declined to where it was indistinguishable from control at 24 h (data not shown).

Mass spectrometric analysis of remifentanil

The remifentanil standard from Glaxo-Wellcome gave a sharp peak at 13.6 min by GC/MS without derivatisation and was >95% pure based on TIC area. Figure 2 presents the full scan electron-impact mass spectrum of this chromatographic peak including structural assignment of major m/z values. Urine from a horse treated with remifentanil and subsequently extracted by SPE yielded a GC/MS peak with the same retention time and mass spectrum (data not shown), suggesting that parent remifentanil was present in the urine sample.

The concentrations of parent remifentanil in the urine samples were estimated using blank urine to which known concentrations of remifentanil had been added. Extracts were analysed by a GC/MS SIM protocol utilising diagnostic ions derived from the major peaks seen in Figure 2, specifically 319, 303, 227, 212, and 168 m/z, with the 168 m/z base peak being utilised for external standardisation. This approach yielded a linear standard curve. Interpolation of data points on this curve revealed

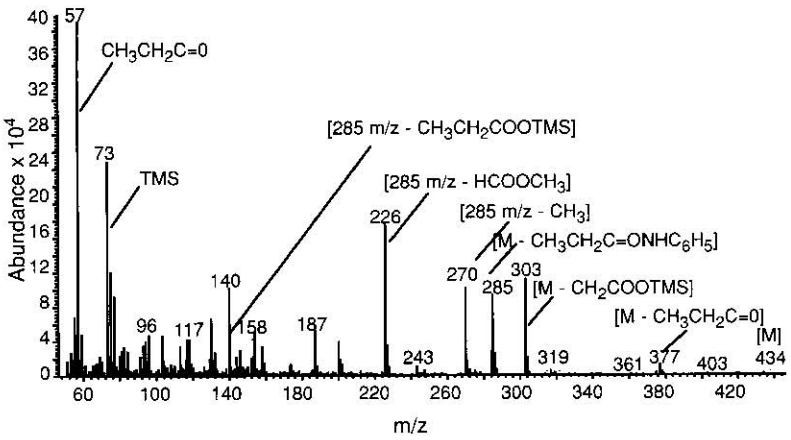


Fig 3: Electron impact mass spectrum of the equine carboxylic acid metabolite as a TMS derivative and observed in an equine urine extract 1 h after remifentanil administration.

that peak urinary concentration was reached 1 h after administration (Table 1), which basically agreed with the ELISA findings. Furthermore, parent remifentanil was detected in urine samples for up to 6 h, also in general agreement with the ELISA data.

Derivatisation of 1 h urine sample extracts with BSTFA + 1%TMCS and analysis by GC/MS revealed a significant peak at 14.10 min retention time that was absent in the control sample. The molecular ion from the corresponding mass spectrum indicated that this 14.10 min peak corresponds in molecular weight to that anticipated for the TMS derivative of a carboxylic acid hydrolysis product of remifentanil. No evidence was found for any other potential metabolites, nor were there any other peaks in the post administration samples that could not be accounted for by comparison to the control samples.

Ion chromatography of selected m/z values derived from full scan GC/MS runs of 1 h urine extracts revealed nested symmetrical peaks within the remifentanil (13.6 min) and remifentanil carboxylic acid TMS derivative (14.1 min) peaks. The relative intensities of the equivalent m/z 57 ions suggested, in the absence of purified standard, that the carboxylic acid metabolite was present at about 10-fold greater concentrations than parent remifentanil. Figure 3 illustrates the full scan mass spectrum of the TMS derivative of the 14.1 min carboxylic acid metabolite including structural interpretation of major peaks. Support for our interpretation of mass spectral peak assignments is provided by the comparability of peak identities with those for parental remifentanil in Figure 2, particularly the 303 m/z peak occurring in either spectrum representing loss of either $CH_2COOTMS$ or CH_2COOCH_3, respectively. Table 2 lists these assignments and demonstrates their excellent correspondence. Table 3 shows that the integrated area for a key diagnostic ion (226 m/z) for the derivatised carboxylic acid of remifentanil has the same excretion profile as that of parent remifentanil (Table 1).

The acid hydrolysis product of remifentanil was derivatised to produce a chromatogram with a single peak, and its mass spectrum provided a perfect match to that of the equine metabolite in Figure 3. Examination of the hydrolysis product on the 6890/5972 GC/MSD verified that the base hydrolysis product retention time matched that of the equine metabolite.

Discussion

Remifentanil at a clinically effective dose would be expected to produce a clear-cut locomotor response, as previously reported for other members of the group of fentanyl-based μ-opiate agonists. Consistent with this expectation, a 5 mg dose of remifentanil produced locomotor responses. This dose therefore has the ability to influence the racing performance of horses and effective screening and confirmation methods are required for forensic control of this agent. This is emphasised by the fact that there have been no reported identifications of remifentanil in post race drug testing in North America, despite its commercial availability since 1996.

Remifentanil administration was readily detectable with a Fentanil Group ELISA kit. The presence of remifentanil equivalents was demonstrated in equine urine for up to 8 h, indicating its suitability for screening.

Remifentanil was also readily detectable by chromatography with our standard GC injection and oven temperature regimens, which are based on a 5%-phenyl-95%-methylpolysiloxane-coated capillary column programmed from 70°C to 280°C at 20°C/min. The drug was amenable to SPE methods, adapted from Worldwide Monitoring assays for fentanyl-related drugs (United Chemical

TABLE 2: Observed mechanisms of mass spectral fragmentation for remifentanil or its proposed 1-piperidine carboxylic acid metabolite as a TMS derivative, where R = CH3 (remifentanil) or TMS (remifentanil carboxylic acid metabolite); M = molecular ion

Loss through fragmentation, or resultant fragment	Assigned remifentanil peak, m/z	Assigned peak of the carboxylic acid metabolite of remifentanil, m/z
$M - OCH_3$	345	403
$M - CH_3CH_2C{=}O$	319	377
$M - CH_2COOR$	303	303
$M - CH_3CH_2C{=}ONHC_6H_5$	227	285
$M - CH_3CH_2C{=}ONHC_6H_5 - CH_3$	212	270
$M - CH_2COOR - HCOOCH_3$	243	243
$M - CH_3CH_2C{=}ONHC_6H_5 - HCOOCH_3$	168	226
$M - CH_3CH_2C{=}ONHC_6H_5 - CH_2COOR$	154	154
$M - CH_3CH_2C{=}ONHC_6H_5 - CH_3CH_2COOR$	140	140
C_6H_5	77	77
$CH_3CH_2C{=}O$	57	57

TABLE 3: Equine urine extracts after remifentanil administration. Mass spectral area count of the proposed remifentanil carboxylic acid metabolite as a function of the number of hours after dosing with 1 mg iv

Time post administration of remifentanil (h)	Area of the remifentanil-carboxylic acid metabolite's 226 m/z peak occurring at 14.1' RT (x 10e4)
0	0
1	305
2	150
4	40
6	30
8	20
24	5

Technologies, Inc. Catalog: Worldwide Monitoring Bonded Phase Extraction Sorbents, 1996). The SPE method worked well on both remifentanil and its carboxylic acid metabolite. Application of these techniques enabled the detection of parent remifentanil in urine 1–6 h after administration (Table 1).

Full scan GC/MS acquisitions were analysed by ion chromatographic screening based on predicted values for metabolites, and this procedure revealed that the major equine metabolite of remifentanil is the same as that previously described in dogs and humans (Michelsen, *et al.* 1996; Hoke *et al.* 1997). Other potential candidate metabolites could not be found by this method, supporting the contention that the carboxylic acid is the primary equine urinary metabolite.

Remifentanil appears to be metabolised rapidly in the horse. Note from the structure of remifentanil (Fig 1) that there are formally 2 alternatives for the generation of a remifentanil carboxylic acid, one that would result in a 1-piperidinepropionic acid metabolite as illustrated, the other a 4-piperidinecarboxy metabolite. Either of these potential metabolites would presumably undergo TMS derivatisation. Comparison of equine metabolism of remifentanil to analogous metabolism described in the literature for man and dogs has enabled selection of the 1-piperidinepropionic acid metabolite from these identical molecular weight carboxylic acid candidates. Further support can be made by mass spectral analysis. Mass Spec Calculator Pro software predicted identical mass spectral peaks for the 2 candidates, with the exception of McLafferty rearrangement products: 88 m/z for the 4-carboxy TMS derivative and 285 m/z for the 1-propionic acid TMS derivative. Since both the equine metabolite and hydrolysis product have significant 285 m/z peaks, it is indirect support that the sterically less-hindered 1-propionic acid methyl ester is more prone both to non-specific esterases *in vivo* and base hydrolysis *in situ*. Because there are 2 potential targets for hydrolysis (Fig 1), it is of interest that chemical hydrolysis produced the same carboxylic acid as did esterases *in vivo*.

Other experiments showed that the integrated area for a key diagnostic ion (226 m/z) for the carboxylic acid of remifentanil exhibits the same excretion profile as that of parent remifentanil. Comparison of the 14.1 min 226 m/z area and its ratio to the 168 m/z 13.6 min area over the 0–24 h sampling period implied a roughly 10-fold preponderance of the carboxylic acid metabolite over parental remifentanil (data not shown). The much higher concentrations of the remifentanil 1-piperidinepropionic acid hydrolysis product suggest that screening and confirmation methods should focus on this urinary metabolite of remifentanil, particularly for forensic samples.

Conjugates of the carboxylic acid metabolite in urine were screened by electrospray-negative mode MS. Sulphate and glucuronide conjugates

were basically excluded by this method, since neither 441 m/z nor 537 m/z predicted peaks were observed. No other potential candidate metabolites could be visualised, supporting the implication that the 1-piperidinepropionic acid hydrolysis product of remifentanil is the primary urinary metabolite in horses. Since the remifentanil-COOH metabolite could be visualised without prior glucuronidase or sulphatase treatment, and since direct screening for such conjugates was negative, we tentatively concluded that the carboxylic acid was excreted directly.

To summarise, a 5 mg iv dose of remifentanil in horses produces clear-cut evidence of locomotor stimulation, consistent with the previously described pharmacological characteristics of such agents. The evidence presented suggests that remifentanil is metabolised in the horse in much the same way as in man and dogs, specifically by hydrolysis to 4-methoxycarbonyl-4-[(1-oxopropyl)phenylamino]-1-piperidinepropionic acid. Remifentanil and its metabolites are readily detected as fentanyl equivalents in urine samples by ELISA-based screening. Confirmation methods that employ extraction, TMS derivatisation and mass spectrometric verification of the major equine urinary metabolite should allow effective control of the unauthorised use of this substance in racing horses.

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DETECTION AND DISPOSITION OF PYRILAMINE AND ITS METABOLITE O-DESMETHYLPYRILAMINE IN EQUINE BLOOD AND URINE: A PRELIMINARY REPORT

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ABSTRACT

Pyrilamine is an antihistamine used in human and equine medicine. Because antihistamines produce central nervous system effects in horses, pyrilamine is classified as an ARCI Class 3 substance, which has the potential to affect the performance of racehorses. The predominant equine urinary metabolite is O-desmethylpyrilamine (O-DMP). The ELISA and GC/MS detection of pyrilamine and/or O-DMP in equine blood and urine samples, following oral and iv administration, are reported.

Pyrilamine and O-DMP are detected readily in equine blood and urine by the pyrilamine ELISA and GC/MS selected ion monitoring (SIM) methods used. After iv injection of pyrilamine (100 mg), the ELISA detected pyrilamine equivalents in serum for up to 11 h and in urine for up to 69 h. We used the GC/MS-SIM method to quantify pyrilamine and O-DMP in equine blood and urine. After iv administration of pyrilamine (300 mg/horse), serum pyrilamine concentrations declined from about 350 ng/ml at 5 min post dose to about 6 ng/ml at 8 h post dose. After oral administration of pyrilamine (300 mg/horse) serum concentrations reached about 20 ng/ml at 1 h post dose, falling to about 0.6 ng/ml at 8 h post dose. No pyrilamine was detected in serum at 24 h post dose by either route.

Recovery of O-DMP from urine was optimised by incubating the urine samples for about 12 h at 37°C with *Helix pomatia* ß-glucuronidase. Hydrolysis was followed by solid phase extraction (SPE), trimethylsilyl derivatisation and GC/MS-SIM analysis to determine the apparent O-DMP concentrations. After iv injection of pyrilamine (300 mg/horse) O-DMP was recovered at a level of about 40 µg/ml at 1 h post dose thereafter declining to about 4 ng/ml at 168 h post dose. After oral administration, the O-DMP recovery peaked at about 28 µg/ml at 6 h post dose and declined to about 3 ng/ml at 168 h post dose.

Preliminary pharmacokinetic analysis of serum pyrilamine concentrations following iv and oral administration suggests relatively low (12%) bioavailability of pyrilamine in horses. However, the apparent urine concentrations of O-DMP after iv and oral administrations were essentially equivalent at 6 h post dose, suggesting rapid oral absorption and extensive first-pass metabolism followed by enterohepatic circulation of this agent.

INTRODUCTION

Pyrilamine (N-[(4-methoxyphenyl)methyl]-N,N-dimethyl-N-2-pyridinyl-1,2-ethanediamine) is an ethylenediamine type antihistamine that is an H_1-receptor antagonist used extensively in human and veterinary medicine for symptomatic relief of allergic reactions. Although the behavioural effects of pyrilamine on the horse are unknown, general side effects of antihistamines include sedation or central nervous system stimulation, depending on the medication, dose and route of administration (Douglas 1985). Pyrilamine is classified by the Association of Racing Commissioners International (ARCI) as a Class 3 agent, and its use in racehorses is prohibited because it may alter their performance. Therefore, methods for detection and identification of antihistamines and/or their metabolites in biological samples from horses are needed for effective control of these agents. Detection of pyrilamine and/or its metabolites in post race samples may lead to significant sanctions against trainers.

Detection and identification of pyrilamine in urine samples has been hampered because the drug is metabolised extensively, and only small amounts of the parent drug are excreted in urine. The need for studies is accentuated by the fact that