Pharmacokinetics and pharmacodynamics of clemastine in healthy horses

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Clemastine is an H_1 antagonist used in certain allergic disorders in humans and tentatively also in horses, although the pharmacology of the drug in this species has not yet been investigated. In the present study we determined basic pharmacokinetic parameters and compared the effect of the drug measured as inhibition of histamine-induced cutaneous wheal formation in six horses.
The most prominent feature of drug disposition after intravenous dose of 50 μg/kg bw was a very rapid initial decline in plasma concentration, followed by a terminal phase with a half-life of 5.4 h. The volume of distribution was large, V_{ss} = 3.8 L/kg, and the total body clearance 0.79 L/h kg. Notably, oral bioavailability was only 3.4%. There was a strong relationship between plasma concentrations and effect. The effect maximum (measured as reduction in histamine-induced cutaneous wheal formation) was 65% (compared with controls where saline was injected) and the effect duration after i.v. dose was approximately 5 h. The effect after oral dose of 200 μg/kg was minor.
The results indicate that clemastine is not appropriate for oral administration to horses because of low bioavailability. When using repeated i.v. administration, the drug has to be administered at least three to four times daily to maintain therapeutic plasma concentrations because of the short half-life. However, if sufficient plasma concentrations are maintained the drug is efficacious in reducing histamine-induced wheal formations.

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INTRODUCTION

H_1 antagonists have classically been used for treatment of hypersensitivity conditions and urticaria, to antagonize the effect of histamine released from degranulating mast cells. In allergic and hypersensitivity reactions allergens crossbind IgE on the surface of mast cells, thereby causing degranulation of the cells. Histamine is one of the inflammatory mediators released together with proteolytic enzymes, prostaglandines, leukotrienes and proinflammatory cytokines. Intradermally injected histamine causes a local oedema (wheal) with a surrounding erythema, visible on white skin (Rang *et al.*, 1999).

Clemastine is an antihistamine, a competitive inhibitor of H_1 receptors, and it has been approved as a drug in human medicine since the 1960s. The indications are, among others, urticaria and eczema of allergic origin. It is an aminoalkylether, a lipid soluble compound that passes the blood/brain barrier and belongs to the first generation of antihistamines. The compound has some anticholinergic properties in addition to its antihistamine activity (Hedstrand, 2002). Only recently, information on the pharmacokinetics of clemastine in humans has been available because of the high potency of the drug and accordingly difficulties in the analysis of drug concentrations in plasma (Schran *et al.*, 1996) and to our knowledge, no pharmacokinetic data from horses have yet been published.

One condition where clemastine could be useful in equine therapy is for the treatment of 'sweet itch', i.e. hypersensitivity to biting insects, mainly *Culicoides* spp. which is the most common cause of allergic dermatitis in the horse (Greiner, 1995). The syndrome is seen to a much higher extent in some breeds; e.g. in Sweden, approximately 25% of imported Icelandic horses are affected during the grazing season (Broström *et al.*, 1987). Histamine seems to be an important mediator of the clinical signs as local administration of the H_1 antagonist chlorpheniramine eliminates allergen-induced wheal formations and reduces leucocyte chemotaxis (Foster *et al.*, 1998).

The aim of the present study was to increase the basic knowledge about the pharmacology of clemastine in the horse. The drug was chosen as it is well known among Swedish veterinarians and available on the Swedish market (as Tavegyl®) both for i.v. and oral administration (Hedstrand, 2002). This drug has also been found to be interesting when controlling canine pruritus (Paradis *et al.*, 1991). Using an experimental model to assess antihistamine activity, plasma concentrations of clemastine were compared with the effect of the drug and basic pharmacokinetic parameters were calculated after i.v. and oral administration of clemastine, respectively.

MATERIALS AND METHODS

Horses

Six adult Standard breds (three geldings and three mares) weighing between 448 and 518 kg were used. For all horses, results of physical examinations performed before the study were normal. None of them had received any medication for at least 4 weeks before the study. They were fed hay and oats when stabled, and spent the daytime outdoors. Water was available *ad libitum*. The study protocol was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

Drug administration

The study had a crossover design and all the six participating horses received clemastine orally and intravenously (three of the horses received the drug first orally then intravenously and the remaining three horses started with the i.v. dose). The washout period between administrations was between 18 and 22 days.

For i.v. dose, clemastine (Tavegyl® solution for injection 1 mg/mL, Novartis, Stockholm, Sweden), 50 μg/kg bodyweight was given diluted in 80 mL of saline solution (0.9% NaCl) and administered into the jugular vein as a constant infusion over 20 min. For oral dose, 200 μg/kg clemastine (Tavegyl® tablets, 1 mg) was dissolved in water and administered via a stomach tube. The tube was rinsed with water before removal. Horses were (with one exception) not fed for at least 10 h before and 4 h after oral drug administration. Results from a pilot study had shown that the doses chosen were adequate to allow quantification of the drug in plasma.

Blood sampling

Blood samples were collected from a jugular vein catheter (not used for the infusion) before, and 5, 10, 15, 20, 25, 30, 40, 50, 60, 75 and 90 min and 2, 3, 4, 6, 10, 22 and 28 h after the start of the i.v. drug infusion. Blood was also collected before and 10, 20, 30, 45, 60, 75 and 90 min and 2, 3, 4, 5, 7 and 11 h after the oral administration. The blood was collected in test tubes with heparin as anticoagulant and was centrifuged at 600 ***g*** for 10 min. Plasma was separated and frozen at −20 °C.

Intradermal testing with histamine

The horses were shaved with an electric clipper on the lateral neck 2 h before the first intradermal injection. Intradermal injections with 7 μg/site histamine hydrochloride (0.1 mg/mL, ex tempore, Apoteket AB, Sweden) were given using 27 gauge needles. After 20 min the diameter of the skin reaction was measured twice using a vernier calliper. Testing was performed at 0.5, 1, 2, 4, 6, 10 and 22 h after i.v. and 0.5, 1, 2, 4, 7, 11 and 23 h after oral administration of clemastine, respectively. Sterile saline (0.07 mL) served as a negative control (a mean value was calculated from 10 administrations).

Recording of other pharmacodynamic effects

The horses were kept under constant observation for at least 2 h during and after drug administration and any clinical sign of drug response was recorded. During, and for 1 h after the i.v. infusion, the heart rate was continuously recorded using a telemetric device (Polar Beat, Polar Electro Oy, Kempele, Finland) fastened with a girth.

Measurement of the plasma protein binding level

Binding of clemastine to equine plasma proteins was determined by means of equilibrium dialysis, using semipermeable membranes (MWCO 12–14 000, Spectrapor, Spectrum Medical Industries Inc, Los Angeles, CA, USA). Plasma samples were adjusted to a pH of 7.4 with 1 M HCl (8–10 μL/mL of plasma), and 1 mL of plasma from each of the six horses was dialysed against 1 mL of buffer solution (28 mM Na_2HPO_4, 5.6 mM NaH_2PO_4 and 38 mM NaCl, pH 7.4) containing 100 ng of clemastine/mL at 37 °C for 4 h. The fraction of drug bound to plasma proteins was calculated as the ratio between the bound fraction (i.e. the difference between drug concentration in plasma and the unbound fraction in the buffer solution) and drug concentration in plasma. Pooled plasma and buffer from two dialysis cells of each were used for the analysis and the experiment was repeated three times for each horse. Results of preliminary tests indicated that equilibrium was reached before 3 h of dialysis and no differences in protein binding were detected for plasma clemastine concentrations ranging from 10 to 300 ng/mL.

Analysis of clemastine concentrations

Chemicals

Clemastine hydrogen fumarate was obtained from Novartis Pharma AG (Basel, Switzerland). Orphenadrine citrate was from Sigma (St Louis, MO, USA). The water was of Millipore quality (Millipore, Bedford, MA, USA). All other reagents were of analytical grade or better and used without further purification. Blank horse plasma was obtained from National Veterinary Institute (SVA, Håtunaholm, Sweden).

Sample pretreatment

The plasma samples and the samples from both compartments of the equilibrium dialysis chambers were pretreated in the same

way: 100 μL of the internal standard orphenadrine (containing 3.4 ng orphenadrine base for the samples from the oral administration, 10.3 ng for the samples from the i.v. administration and the protein binding study), 4 mL of hexane:dichloromethane 4:6 and 1 mL of 0.1 M NaOH were added to 1 mL of each plasma sample or each sample from the equilibrium dialysis study. The samples were mixed for 15 min and centrifuged for 10 min at 2300 ***g***. The supernatant was removed and evaporated to dryness under a stream of nitrogen at ~60 °C. The residues were reconstituted in 100 μL of 40% methanol and 60% 0.1 M acetic acid in water.

Calibration and validation

Standards for the calibration curve were prepared by adding varying amounts of clemastine to blank horse plasma, yielding concentrations of 0.058, 0.114, 0.235, 0.470, 0.58, 1.45, 4.7, 5.8, 11.4 and 114 ng base/mL. The calibration curve was constructed by linear regression of peak-area ratios of clemastine to internal standard as a function of clemastine concentration. Different concentration intervals in the calibration curve were used for different samples (see Table 1). Quality control (QC) samples were prepared by spiking blank horse plasma at the concentrations 0.175, 1.00, 8.8 and 43.8 ng clemastine/mL, for evaluation of the accuracy and precision of the method. Two QC concentrations were used for each sample series (see Table 1). The standards and the QC samples were treated in the same way as the samples (see above).

Liquid chromatography tandem mass spectrometry

The reconstituted samples were quantified with liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS) using an HP1100 liquid chromatograph with a binary pump (Hewlett-Packard, Waldbronn, Germany) and a Luna $C_{18}(2)$ (Phenomenex, Torrance, CA, USA) chromatographic column (length 150 mm, inner diameter 2.00 mm and particle diameter 5 μm). The samples were eluted using a 10-min gradient of 50–100% methanol in 0.05% formic acid in water. The injection volume was 5.0 μL and the volumetric flow rate 0.2 mL/min. The chromatography was performed at ambient temperature.

A Quattro LC (Micromass, Manchester, UK) quadrupole-hexapole-quadrupole mass spectrometer with an electrospray interface (ESI) was connected to the column outlet. The software MASSLYNX (version 3.3) was used for instrument control and data acquisition. The mass spectrometric parameters were optimized manually during direct infusion of a 1-mg/mL solution of clemastine fumarate. The solution was infused with a syringe pump (flow rate 2 μL/min) through a connecting T where it was mixed with the liquid chromatography (LC) mobile phase (flow rate 0.2 mL/min). The optimized parameter settings were: capillary voltage +2.92 kV, cone +15 V, extractor +7 V and RF lens +0.9 V. The desolvation temperature was 350 °C and the source block had a temperature of 130 °C. The desolvation gas flow was 912 L/h and the nebulizer gas flow was 102 L/h. Argon at a pressure of 7.6×10^{-4} mbar filled the hexapole collision cell. The mass spectrometer was run in multiple reaction monitoring mode, switching between the transitions m/z 344 → 215 for clemastine and 270 → 181 for orphenadrine.

Pharmacokinetic calculations

The measured plasma concentrations of clemastine were plotted vs. time for each horse and data were analysed using the computer software WinNonlin (version 3.0, Pharsight corporation, Mountain View, CA, USA) with its incorporated equations for pharmacokinetic analysis. Different models and weighting factors were assessed by visual inspection of the curvefits and the residuals' scatter plots, together with the goodness of fit measures incorporated in the software (including the Akaike and Schwartz criteria).

The oral bioavailability (F) was defined as

$$F = (AUC_{\text{p.o.}}/AUC_{\text{i.v.}}) \times (\text{Dose}_{\text{i.v}}/\text{Dose}_{\text{p.o}})$$

Table 1. Accuracy, precision (RSD) and linearity data of clemastine

	Quality control (QC) sample level (ng/mL)	Accuracy (%)	RSD % (n)	Calibration curve (ng/mL)	Correlation
A. Intravenous administration					
Samples from horses 1–3	1.00	121	2.19 (5)	0.47–14.5	0.9722
	43.8	82	11.1 (5)	0.47–114	0.9963
Samples from horses 4–6	1.00	70	22.8 (10)	0.47–14.5	0.9979
	43.8	87	11.8 (10)	0.47–114	0.9924
B. Oral administration					
0–3-h postadministration	0.175	104	7.80 (10)	0.058–11.4	0.9969
	1.00	84	5.87 (10)	0.058–11.4	0.9969
4–11-h postadministration	0.175	112	9.25 (10)	0.058–1.45	0.9893
	1.00	122	13.5 (10)	0.058–1.45	0.9893
C. Protein-binding study					
Plasma compartment	8.8	90	7.74 (10)	0.47–114	0.9924
Buffer compartment	8.8	91	6.93 (10)	0.47–14.5	0.9957

Pharmacodynamic calculations

Wheal areas were calculated from the mean value of the two diameters measured and expressed as percentage inhibition of wheal formation. For each horse, 0% inhibition was defined as the wheal area after histamine injection only (before administration of clemastine) and 100% as the wheal area after injection of sterile saline (mean value of 10 replicates). For data where compartmental pharmacokinetic parameters could be estimated, the effect over time profile was analysed using a pharmacokinetic/pharmacodynamic link model incorporated in the WinNonlin software. The concentration/response equation used was:

$$E = (E_{\max.} \times C_e^{\gamma})/(C_e^{\gamma} + EC_{50}^{\gamma})$$

where C_e is the apparent effect compartment concentration and EC_{50} is the plasma concentration producing 50% of the maximal effect. Gamma (γ) is the exponent expressing the sigmoidicity of the concentration–effect relationship. Using this model, E_{max}, EC_{50} and K_{e0} (the rate constant for the elimination of drug from the effect compartment) were calculated (Toutain *et al.*, 1994).

RESULTS

Validation of the analytical method

The plasma samples and the samples from the protein binding study were divided into six groups analysed on different occasions. QC samples, prepared by spiking blank plasma with clemastine, were analysed together with each group. The obtained accuracy, precision and linearity are presented in Table 1.

Pharmacokinetics

It was possible to determine the concentration of clemastine in plasma for 22–28 h after i.v. administration. In all individuals investigated, the plasma concentrations following the termination of the infusion declined rapidly, followed by two different consecutive phases with less steep appearance (Fig. 1). The plasma concentration vs. time profile was most adequately described as a three-compartment pharmacokinetic model (Fig. 2) using the equation:

$$C(t) = I_1(e^{-\lambda 1 t} - e^{-\lambda 1 t^*}) + I_2(e^{-\lambda 2 t} - e^{-\lambda 2 t^*}) + I_3(e^{-\lambda 3 t} - e^{-\lambda 3 t^*})$$

where t^* (the length of the infusion) is included to create a factor that compensates for the time delay in relation to an i.v. bolus model. The pharmacokinetic parameters were calculated from the y axis intercepts (I_{1-3}) and the exponents of the respective phase (λ_{1-3}). The volume of the central compartment (V_1) and the rate constants K_{21} and K_{31} were used to estimate the intercepts. The appropriate weighting factor was found to be 1/Y. The main pharmacokinetic parameters obtained from i.v. data are presented in Table 2.

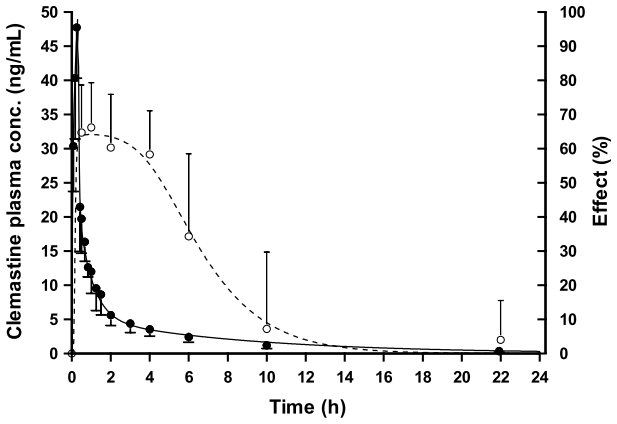

Fig. 1. Plasma concentrations vs. effect after i.v. dose of 50 μg/kg clemastine. The solid symbols represent plasma concentrations (mean ± SD, $n = 6$) and the open symbols percentage inhibition of histamine-induced wheals (mean ± SD, $n = 6$). The curve fits represent a three-compartment pharmacokinetic model and a pharmacodynamic link model, respectively.

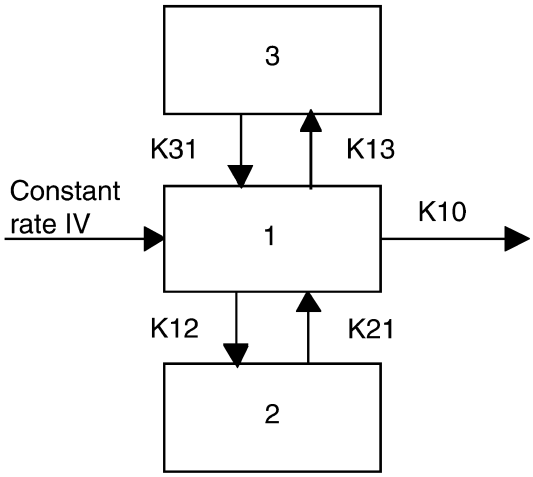

Fig. 2. The three-compartment pharmacokinetic model with constant rate infusion (from the WinNonlin model library).

Table 2. Pharmacokinetic parameters calculated from drug plasma concentrations after i.v. dose of 50 μg/kg bw clemastine ($n = 6$). For an explanation of the abbreviations, see 'Materials and methods'

Parameter	Median	Range
C_{max} (ng/mL)	55	37–62
V_1 (L/kg)	0.15	0.12–0.23
K_{21} (h^{-1})	3.7	2.2–5.9
K_{31} (h^{-1})	0.30	0.24–0.36
λ_1 (h^{-1})	29	23–42
λ_2 (h^{-1})	1.4	0.82–1.9
λ_3 (h^{-1})	0.13	0.11–0.15
AUC (μg h/L)	63	53–92
Cl (L/h kg)	0.79	0.55–0.89
V_{ss} (L/kg)	3.8	2.5–4.9
I_1 (ng/mL)	300	170–400
I_2 (ng/mL)	20	14–46
I_3 (ng/mL)	4.9	3.9–8.4
$t_{1/2\ \lambda 1}$ (h)	0.024	0.017–0.030
$t_{1/2\ \lambda 2}$ (h)	0.49	0.36–0.84
$t_{1/2\ \lambda 3}$ (h)	5.4	4.5–6.4

After oral administration, no such prominent distribution phase was seen, although the absorption was rapid with no apparent lag time (Fig. 3, Table 3). A noncompartment model was found most appropriate to describe the plasma concentration vs. time profile after oral administration, using the linear trapezoidal rule to calculate the area under the curve (*AUC*). To extrapolate the *AUC* to infinity, the terminal slope (λ_z), from 2 h, of the log concentration–time profile was used. The fraction of *AUC* and fraction of the area under the first moment curve (*AUMC*) extrapolated (median with range in brackets) were 6.7% (3.0–11) and 23% (11–34) and the correlation coefficient for λ was 0.95 (0.90–0.99). The $t_{½}$ connected to the terminal (λ_z) phase was intermediate to the λ_2 and λ_3 half-lives after i.v. administration (Table 3).

One horse was excluded from the summation of data after oral administration as it was difficult to define the λ phase. This horse was fed hay before the drug administration and the absorption of the drug might have been delayed. In this horse $t_{½\lambda z}$ was considerably longer and the fraction of *AUC* and AUMC large. The correlation coefficient for λ was only 0.69.

The plasma protein binding was 99% in all individuals ($n = 6$).

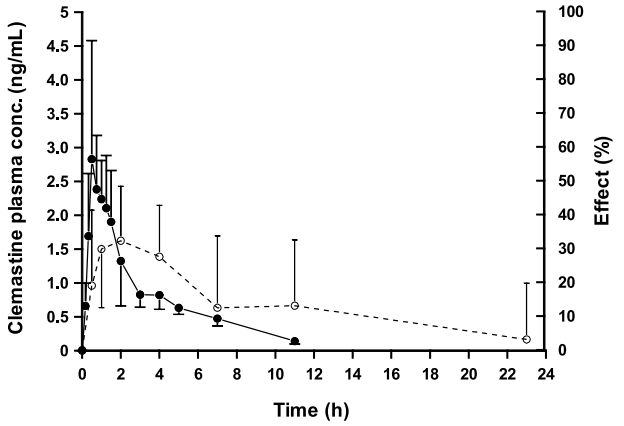

Fig. 3. Plasma concentrations vs. effect after oral dose of 200 μg/kg clemastine. The solid symbols represent plasma concentrations (mean ± SD, $n = 5$) and the open symbols percentage inhibition of histamine-induced wheals (mean ± SD, $n = 6$). Zero percentage and 100% effect correspond to wheal areas of 288 ± 37.3 and 53.1 ± 4.5 mm^2, respectively.

Table 3. Pharmacokinetic parameters calculated from drug plasma concentrations after oral dose of 200 μg/kg bw clemastine ($n = 5$). For an explanation of the abbreviations, see 'Materials and methods'

Parameter	Median	Range
C_{max} (ng/mL)	2.7	1.9–5.9
T_{max} (h)	1.0	0.50–1.50
AUC (μg h/L)	8.5	7.7–11.5
λ_z (h^{-1})	0.24	0.20–0.32
$t_{1/2\ \lambda z}$ (h)	2.9	2.2–3.4
F (%)	3.4	2.1–5.0

Pharmacodynamics

No adverse effects were seen after the treatment. The heart rate remained within normal limits throughout the observation period.

The inhibition of histamine-induced wheal areas was most prominent after i.v. drug administration, where it was possible to link the pharmacodynamic effect vs. time profile to the three-compartment pharmacokinetic model (Table 4, Fig. 1). The effect peaked at 65% at C_{max} and remained over 50% for approximately 5 h.

The effect duration after oral administration was considerably shorter with a maximum effect of only approximately 30%, i.e. half of the E_{max} according to the PK/PD link model. The C_{max} after oral administration was equal to the calculated EC_{50}-value (Table 4).

DISCUSSION

Various kinds of species differences are confounding factors in veterinary pharmacology, limiting the possibilities to extrapolate doses and treatment protocols between different kinds of animals. Most often, these differences concern pharmacokinetics, as both body composition and metabolic rates might vary. Regarding H_1 antagonists, pharmacokinetic data in different species are scarce (Plumb, 1999). Obviously, there is a demand for studies on H_1 antagonists in veterinary practice and therefore, we present basic pharmacokinetic and pharmacodynamic data for one such drug: clemastine.

To our knowledge, no comprehensive pharmacokinetic studies with clemastine have yet been published, regardless of species, as the high potency of the drug has limited the possibilities to quantify the drug in plasma. In the past different analytical methods have been used such as a radioimmuno assay (Schran *et al.*, 1996) and gas chromatography with nitrogen–phosphorus detection (Davydova *et al.*, 2000). In the present study, a more selective analytical method was used based on liquid chromatography tandem mass spectrometry (LC-MS/MS).

The ideal internal standard, i.e. deuterated clemastine, was unfortunately not available and the structurally related compound orphenadrine was chosen instead (Davydova *et al.*, 2000). Fluctuating conditions in the extraction and LC/MS/MS analyses might therefore have affected clemastine and the internal standard to a different extent, yielding the somewhat low precision for some of the sample series. However, accuracy

Table 4. Results obtained from the PK/PD link model. The parameters were calculated from the mean values ($n = 6$) of drug plasma concentrations and effect at each time point

Parameter	Result
E_{max} (%)	65
EC_{50} (ng/mL)	3.3
λ	3.6
K_{e0} (h^{-1})	0.71
$t_{1/2\ Ke0}$ (h)	0.98

and precision were significantly improved by using a shorter concentration interval in the calibration curve for the low level samples. Thus, the method performance was considered sufficiently good for the conclusions drawn in the PK/PD and protein binding studies discussed below.

In humans, some basic parameters are available but these are obtained only after oral administration of the drug. Schran *et al.* (1996) report about a rather long absorption time in humans and that two different disposition half-lives of 5 and 21 h, respectively, were detected. In horses, the absorption after oral administration was rapid and also the elimination of the drug was much faster than in humans. The half-life was only 3 h. Data after i.v. administration could be fitted into a three-compartment model indicating that the elimination of the drug is also biphasic in horses, although the time course is much more rapid than in humans with half-lives of only 30 min and 5.4 h for the λ_2 and λ_3 phase, respectively. Obviously, the latter phase was detected only partly after oral administration because of the shorter time course during which the drug was detected in plasma after oral compared with i.v. administration.

Although the dose per kg bw given orally was much higher than the dose recommended for humans (25 μg/kg BID & Hedstrand, 2002), the plasma concentration hardly reached therapeutic levels in horses. The oral bioavailability was only between 2 and 5%, considerably lower than the 20–70% estimated for humans and the clearance was higher (Schran *et al.*, 1996). The data are not really comparable as the human data are estimated only from oral administration of the drug, but it seems very likely that the metabolic rate is much higher in horses. Clemastine is extensively metabolized in the body. After oral dose in humans no parent compound was found in urine indicating that metabolism is the main route of elimination. Mono-oxidation is a part of the pathway of metabolism and thus cytochrome P450 (CYP) is likely to be involved (Choi *et al.*, 1999). *In vitro* studies show that clemastine is a substrate for and also an inhibitor of CYP 2D6 (Hamelin *et al.*, 1998). The activity of CYP 2D6 in the liver is much higher in horses than in humans (Chauret *et al.*, 1997). Hence, metabolism by CYP 2D6 of clemastine in horses could possibly explain the low oral bioavailability and the high clearance.

Clemastine has been extensively used in humans for several decades but the published documentation of its safety and efficacy is scarce. There are few scientific publications reporting on experimental pharmacodynamics of the drug and available papers report on studies where clemastine has been used as a pharmacological tool in comparison with other drugs (e.g. Thomas *et al.*, 1985; Abila *et al.*, 1994). Novartis – the drug company marketing Tavegyl®, a formulation which has clemastine as the active compound – reports from their data on file that the effect duration for Tavegyl® is 12 h with an effect maximum 5–7 h after oral administration (Hedstrand, 2002). The effect was (similar to that in the present study) measured as inhibition of wheal formation after intracutaneous histamine provocation. From these data a twice-daily dosage regimen is recommended for repeated administration to humans. The effect duration after i.v. infusion to horses, where the onset of effect was not delayed by absorption, was considerably shorter, only approximately 5 h in this study although the dose used was high (C_{max} was 55 μg/L kg which is approximately 10 times the lowest concentration that gave maximal effect). This indicates that to maintain plasma concentrations within the therapeutic window a considerably shorter dosage interval would be appropriate to horses when compared with horses. It would, however, be inconvenient to administer the drug that often and therefore clemastine does not seem to be suitable for repeated administration to horses, unless a slow-release formulation is available. Until then, clemastine can be useful only in treatment where the rapid onset of the effect is important and i.v. infusion/slow injection can be used.

In conclusion, the present data show that the pharmacokinetics of clemastine in horses limit the options to use this drug. Due to the low oral bioavailability and the rapid disposition of the drug, clemastine cannot be recommended for control of hypersensitivity conditions in horses with repeated administration of an antihistamine. Parenteral treatment is a prerequisite if therapeutic plasma concentrations are to be obtained; the dosage interval would, however, be unreasonably short. However, the inhibitory effect on histamine-induced wheals was prominent and there was a good relationship between plasma concentrations and effect.

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