Oral and intravenous administration of nimesulide in the horse: rational dosage regimen from pharmacokinetic and pharmacodynamic data

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Keywords: horse; nimesulide; NSAIDs; COX-2-inhibitors; pharmacokinetics; pharmacodynamics; oral administration; intravenous administration; bioavailability; protein binding

Summary

Reasons for performing study: The selective COX-2-inhibitor nimesulide is used extra-label in equine veterinary practice as an anti-inflammatory agent. However, there are no data on which to base the rational use of the drug in this species. Objectives: To determine the effective COX selectivity of nimesulide in the horse, and suggest a suitable dosing schedule. Methods: The pharmacokinetics of nimesulide in the horse after oral administration (1 mg/kg bwt), and oral and i.v. administration (1.5 mg/kg bwt) were investigated, effects of feeding status on bioavailability determined, and plasma protein binding of the drug and its principal metabolites measured. Compartmental and noncompartmental pharmacokinetic analyses were performed. The plasma concentration-time profile was used, together with in vitro literature data on nimesulide inhibition of COX isoforms, to determine the effective COX selectivity of nimesulide in the horse, and suggest a suitable dosing schedule. Results and Conclusions: The findings suggest that 1.5 mg/kg bwt may produce adequate clinical effects and that the dosing interval should be 12–24 h depending on condition severity. However, at that dose, the concentration in the animal exceeds the in vitro IC50 for both isoforms, so that COX-1/COX-2 selectivity is lost and side-effects due to COX-1 inhibition are a possibility. Nimesulide should therefore be used with caution in equine clinical practice.

Introduction

Subsequent to the discovery of the 2 main cyclooxygenase (COX) isoforms in mammals (Vane et al. 1998) the view developed that nonsteroidal anti-inflammatory drugs (NSAIDs) that selectively inhibited the inducible COX-2 isofor, but had reduced inhibitory effects on the constitutive COX-1 isofor, would have fewer gastrointestinal side-effects than substances that strongly inhibited COX-1 (Vane and Botting 1998; Cannon 1999; Kaplan-Machlis and Klostermeyer 1999). New selective COX-2 inhibitors were, therefore, developed (Talley 1999) and available NSAIDs classified according to their relative abilities to inhibit COX-1 and COX-2 in man and animals of veterinary interest. It is now clear, however, that selective COX-2 inhibitors are not necessarily safer or more effective anti-inflammatory agents than conventional NSAIDs (Feldman and McMahon 2000; Kam and See 2000; Wallace 2002). For example, highly selective COX-2 inhibitors have reduced gastrointestinal side-effects in man, but are also associated with increased frequency of severe cardiovascular adverse events (Psaty and Furberg 2005; Vonkeman et al. 2006). Adverse vascular effects have also been recently documented with high doses of the classic NSAIDs ibuprofen and diclofenac (Kearney et al. 2006).

NSAIDs are used extensively in equine veterinary practice. It has been suggested that selective COX-2 inhibitors may be preferable to nonselective COX inhibitors in the management of equine colic, as they seem to have fewer adverse effects on dorsal and ventral colon motility and may not hinder the repair of ischemically injured jejunal mucosa (Campbell and Blikslager 2000; van Hoogmoed et al. 2002). However, selective COX-2 inhibitors can slow the healing of existing ulcers (Moreau et al. 2005) and horses, particularly racehorses, are prone to asymptomatic gastrointestinal ulcers (Dionne et al. 2003).

It is, therefore, important to determine the COX selectivity of individual NSAIDs (expressed as the ratio of COX-1 and COX-2 IC50s in blood after treatment) used in equine clinical practice. In fact Lees et al. (2004a,b) advocated integration of pharmacodynamic (PD) with pharmacokinetic (PK) data, in order to obtain information for the rational use of NSAIDs in veterinary practice. Such studies provide information on (a) species-specific activity, (b) the dose necessary to produce an adequate anti-inflammatory effect and (c) indications of the risks of unwanted side effects.

Nimesulide is a sulphonanilide derivative shown to have anti-inflammatory, analgesic and antipyretic effects in animal
exercised. Water was supplied and animals were fed a small amount of hay about 4 h after drug administration. All animals of individual stalls throughout the study and received no medication. 3 females, weight 390–520 kg. The animals were housed in 15 healthy horses age 3–6 years were divided into 3 Groups. Group A, 4 Thoroughbreds, 2 males and 2 females, weight 385–466 kg; Group B, 4 Thoroughbreds, 2 males and 2 females, weight 410–550 kg, and Group C, 7 Thoroughbreds, 4 males and 3 females, weight 390–520 kg. The animals were housed in individual stalls throughout the study and received no medication in the 30 days leading up to drug administration. Animals of Groups A and B were fasted for 12 h before drug administration, while Group C had free access to food (pasture and hay). Fasted horses up to 24 h after treatment when they urinated spontaneously.

Materials and methods

The study was carried out in compliance with National Legislation (DL n. 116, 27th January 1992) deriving from Directive N.86/609/CEE 24th November 1986 on the protection of Animals used for Experimental and other Scientific purposes, and all the animal procedures were approved by the local Ethics Committee.

Animals

Fifteen healthy horses age 3–6 years were divided into 3 Groups. Group A received nimesulide orally and i.v. at 1 mg/kg bwt; Groups B and C received nimesulide orally, once only at 1 mg/kg bwt. For oral administration the granular preparation was mixed with a small quantity of oats and administered under veterinary surveillance. The preparation was always totally consumed in a few minutes. For i.v. administration a venous catheter was used and the solution injected slowly as single bolus.

Sampling

Blood samples were collected into nonheparinised tubes before administration (time 0) and at various subsequent times between 0.08 h and 24 h, depending on administration route. Sampling times (h) were:

- **Group A** i.v.: 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24
- **Group A and B** orally: 0.33, 0.67, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24
- **Group C** orally: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24.

Drugs

For i.v. administration, nimesulide1 was dissolved in aqueous sodium hydroxide (0.1 mol/l) and the pH adjusted to 9.5 (± 0.2) with hydrochloric acid (0.1 mol/l). The solution was prepared on the day of administration, and was filtered through a disposable 0.22 μm filter (Acrodisc PF)2 before injection. The final nimesulide concentration, assayed by validated HPLC method, was 19.8 mg/ml. For oral administration a commercial granular preparation (Aulin)3 was used.

Experimental design

Horses of **Group A** were administered nimesulide according to an i.v./oral cross-over design with a 2-week interval between administrations. Horses of **Groups B** (fasted) and **C** (not fasted) were treated once orally.

Drug administration

Since nimesulide is not authorised for use in horses, doses were based on those used in man. The typical oral dose in man is 1.4–2.8 mg/kg bwt; and the conservative oral dose of 1 mg/kg bwt was used initially. Subsequently, oral and i.v. doses of 1.5 mg/kg bwt were used following lack of observed adverse effects at 1 mg/kg bwt. **Group A** received nimesulide orally and i.v. at 1.5 mg/kg bwt; **Groups B** and **C** received nimesulide orally, once only at 1 mg/kg bwt.

For oral administration the granular preparation was mixed with a small quantity of oats and administered under veterinary surveillance. The preparation was always totally consumed in a few minutes. For i.v. administration a venous catheter was used and the solution injected slowly as single bolus.

Assays

Nimesulide and its metabolites 4-hydroxynimesulide (4-HXN) and 4-amino-2-phenoxymethanesulfonanilide (4-APMS) for analysis were supplied by Helsinn Chemicals, Lugano, Switzerland. Serum and urine concentrations, 4-HXN and 4-APMS were determined by HPLC using external standard calibration. Nimesulide and metabolites were extracted into chloroform: 0.2 ml of serum or
urine was added to 0.5 ml borate buffer (0.56 mol/l; pH 9) and shaken with 8 ml chloroform. The HPLC method used a reverse-phase column and isocratic conditions (Inertsil5 150-5 ODS-2, 5 µm, 250 x 4.6 mm). The mobile phase was: (A) 25 mmol/l of potassium dihydrogen phosphate and triethylamine 1 mmol/l, adjusted to pH 7.5 (± 0.1) with potassium hydroxide 10 mol/l; (B) acetonitrile plus buffer A, ratio 58:42 v:v. All runs were performed at a flow rate of 1 ml/min; run times were 11 min. A UV LC90 detector was used, set to read at 230 nm.

Protein binding

Serum protein binding was determined in vitro for nimesulide, 4-HXN and 4-APMS in the concentration range 0.5–10 µg/ml. The free compounds were separated by ultrafiltration (Villa et al. 1994, 1997) using a disposable device (Amicon) and analysed by HPLC as described above.

Pharmacokinetics

Pharmacokinetic parameters were deduced from serum concentration-time data using the WinNonLin 4.0.1 software which allows compartmental and noncompartmental analyses of the experimental data. Minimum information criterion estimates (MAICE; Yamaoka et al. 1978) were used to choose the model best fitting the data (Groups A and B). All data points were weighted by the inverse square of the fitted value.

Serum concentrations of nimesulide after i.v. administration were fitted to the following bi-exponential equation:

\[ C(t) = Y_1 \exp(-\lambda_1t) + Y_2 \exp(-\lambda_2t) \]

where \( C_t (\mu g/ml) \) is serum drug concentration at time \( t \); \( Y_1 \) and \( Y_2 \) are serum concentrations extrapolated to time zero of the nimesulide distribution and elimination phases, respectively; \( \lambda_1 \) and \( \lambda_2 \) are the slopes of the distribution and elimination phases of the drug, respectively. The distribution half-time \((t_{1/2d})\) and terminal half-time \((t_{1/2e})\) were calculated as \( \log_2 2/\lambda \); serum concentration at time 0 \((C_0)\) was calculated as the sum of the intercepts.

The volume of distribution \( V_c \) in the central compartment was calculated as:

\[ V_c = \text{Dose}/C_0 \]

The area under the serum concentration-time curve (AUC) and area under the first moment curve (AUMC) were calculated by the method arithmetic trapezes with extrapolation to infinity as follows:

\[ AUC_{(0-n)} = C_{last}/\lambda_2 \]
\[ AUMC_{(0-n)} = tC_{last}/\lambda_2 + C_{last}/\lambda_2^2 \]

where \( t_{last} \) is the last time with measurable concentration \((C_{last})\) and \( \lambda_2 \) is the rate constant for the elimination phase. Mean residence time (MRT), mean absorption time (MAT), body clearance \((C_l)\) and volume of distribution at steady state \((V_{ds})\) were determined from the following equations (Gibaldi and Perrier 1982):

\[ MRT = \text{AUMC/AUC} \]
\[ MAT = \text{MRT}_{oral} - \text{MRT}_{i,v} \]
\[ Cl_l = \text{Dose}/\text{AUC} \]
\[ V_{ds} = Cl \times MRT \]

After oral administration data were fitted to the equation:

\[ C(t) = FD \times \frac{\lambda_3 + e^{-\lambda_4 t}}{\lambda_3 \times \lambda_4} \]

where D is the dose, F the bioavailability, V the volume of distribution, and \( \lambda_3 \) and \( \lambda_4 \) the first order rate constants for the absorption and elimination phases, respectively. Half-times were calculated as \( \log_2 2/\lambda \).

The bioavailability (F%) of nimesulide after oral administration for both doses was calculated as the ratio of the area under serum concentration-time curves \((AUC_{(0-n)})\) after oral and i.v. administration:

\[ F\% = \left( \frac{\text{AUC}_{oral}}{\text{AUC}_{i,v}} \right) \times 100 \]

Statistical analysis

Pharmacokinetic parameters are reported as means with s.d., harmonic means were calculated for terminal half-lives, and pseudostandard deviations (SE) were calculated using a jack-knife technique (Lam et al. 1985). InStat 3.0 was used to perform the analyses. \( c_{max}, T_{max}, \text{AUC} \) and \( MRT \) values for nimesulide after oral administration of 1.5 mg/kg bwt in fasted and 1 mg/kg bwt in fasted and fed conditions were compared by one-way ANOVA with Bonferroni test for multiple comparisons. Differences with \( P<0.05 \) were considered significant. Terminal half-lives after oral administration of

Fig 1: Semi-logarithmic plot of individual nimesulide concentrations in horses of Group A after i.v. (a) and oral administration (b) at 1.5 mg/kg bwt.
1 and 1.5 mg/kg bwt, and terminal half-lives, AUC and MRT after oral and i.v. administration of 1.5 mg/kg bwt were compared by \textit{unpaired} \( t \) test with Welch correction (variances unequal). Differences with \( P<0.05 \) were considered significant.

**Results**

**Serum and urine concentrations**

HPLC retention times for 4-HXN, nimesulide and 4-APMS were about 4.3, 7.0 and 8.1 min, respectively. The analytical method was subjected to intralaboratory validation and found to be specific, linear (in the range 0.05–15 µg/ml), precise and accurate, with a limit of quantification of 0.05 µg/ml for all the compounds investigated in serum and urines. Nimesulide was detected in serum at all sampling times, the 4-HXN metabolite was detected only as non-quantifiable traces and 4-APMS was never detected. Pharmacokinetic analysis was performed for nimesulide only.

Serum concentrations of nimesulide after i.v. and oral administration (1.5 mg/kg bwt) to individual animals in Group A are shown in Figure 1. Serum concentrations after oral administration (1 mg/kg bwt) to individual animals of Group B are shown in Figure 2.

After i.v. administration (1.5 mg/kg bwt, Group A) mean nimesulide concentration (± s.d.) in serum was 13.35 ± 1.05 µg/ml at first sampling (0.08 h), decreased to 9.93 ± 0.68 µg/ml at 2 h post treatment, and subsequently declined more slowly to reach 0.83 ± 0.21 µg/ml at last sampling (24 h).

Following oral administration at 1.5 mg/kg bwt to Group A (fasted), peak concentrations (5.34 ± 1.11 µg/ml) were obtained between 3 and 4 h, and declined slowly to 0.81 ± 0.21 µg/ml at 24 h.

After oral administration at 1 mg/kg bwt to Group B (fasted), peak serum concentration (2.86 ± 0.94 µg/ml) was reached between 4 and 5 h and then declined slowly to 0.38 ± 0.05 µg/ml at 24 h.

Oral administration of 1 mg/kg bwt nimesulide to Group C (fed horses) resulted in marked inter- and intraindividual variability (Fig 3). Peak serum concentration (1.19 ± 0.20 µg/ml) was reached between 7 and 12 h later. At 24 h post dosing only 4/7 horses had serum concentrations above the limit of quantification (mean 0.24 ± 0.97 µg/ml).

**TABLE 1: Total amounts (mg) of nimesulide (NIM) and 4-amino-2-phenoxymethanesulfonanilide (4-APMS) recorded in urine collected after spontaneous urination from horses administered 1 mg/kg bwt of nimesulide orally (Group B)**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Horse 1</th>
<th>Horse 2</th>
<th>Horse 3</th>
<th>Horse 4</th>
<th>Mean ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2h</td>
<td>0.23</td>
<td>0.22</td>
<td>0.29</td>
<td>0.26</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>2–4h</td>
<td>0.23</td>
<td>0.65</td>
<td>0.77</td>
<td>1.63</td>
<td>0.87 ± 1.78</td>
</tr>
<tr>
<td>4–6h</td>
<td>0.45</td>
<td>1.10</td>
<td>0.65</td>
<td>2.48</td>
<td>0.68 ± 1.35</td>
</tr>
<tr>
<td>6–8h</td>
<td>0.30</td>
<td>0.97</td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>8–12h</td>
<td>0.50</td>
<td>1.43</td>
<td>0.59</td>
<td>5.67</td>
<td>1.11 ± 4.38</td>
</tr>
<tr>
<td>12–24h</td>
<td>3.04</td>
<td>12.44</td>
<td>0.20</td>
<td>0.28</td>
<td>0.30 ± 0.68</td>
</tr>
<tr>
<td>Totals</td>
<td>3.04</td>
<td>12.44</td>
<td>1.91</td>
<td>4.65</td>
<td>2.53 ± 2.53</td>
</tr>
</tbody>
</table>

**TABLE 2: Pharmacokinetic parameters (mean ± s.d.) after i.v. and oral administration in the horse (compartmental analysis) Groups A and B**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n = 4, fasted)</th>
<th>Group B (n = 4, fasted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(0–∞) (h.µg/ml)</td>
<td>110.09 ± 10.16 a</td>
<td>59.76 ± 11.29² a, b</td>
</tr>
<tr>
<td>t(_{1/2,1}) (h)</td>
<td>0.73 ± 1.07</td>
<td>6.32 ± 0.91²</td>
</tr>
<tr>
<td>t(_{1/2,2}) (h)</td>
<td>0.68 ± 0.22²</td>
<td>1.20 ± 0.28²</td>
</tr>
<tr>
<td>t(_{1/2,3}) (h)</td>
<td>13.42 ± 0.82</td>
<td>7.63 ± 1.94²</td>
</tr>
<tr>
<td>t(_{1/2,4}) (h)</td>
<td>13.71 ± 1.20</td>
<td>6.80 ± 1.46²</td>
</tr>
<tr>
<td>C(_0) (µg/ml)</td>
<td>5.34 ± 2.88</td>
<td>3.54 ± 1.11</td>
</tr>
<tr>
<td>V(_c) (ml/kg bwt)</td>
<td>54.25 ± 8.82</td>
<td>2.86 ± 0.94</td>
</tr>
<tr>
<td>Cl(_B) (ml/h/kg bwt)</td>
<td>6.57 ± 2.05</td>
<td>4.25 ± 0.50</td>
</tr>
<tr>
<td>AUC after t(_{last}) (%)</td>
<td>54.25 ± 8.82</td>
<td>2.86 ± 0.94</td>
</tr>
<tr>
<td>MRT(0–∞) (h)</td>
<td>6.54 ± 1.95²</td>
<td>18.90 ± 5.88²</td>
</tr>
<tr>
<td>V(_dss) (ml/kg bwt)</td>
<td>6.57 ± 1.95²</td>
<td>18.90 ± 5.88²</td>
</tr>
<tr>
<td>MAT (h)</td>
<td>54.25 ± 8.82</td>
<td>2.86 ± 0.94</td>
</tr>
<tr>
<td>F %</td>
<td>6.57 ± 2.05</td>
<td>4.25 ± 0.50</td>
</tr>
<tr>
<td>C(_{max}) (µg/ml)</td>
<td>54.25 ± 8.82</td>
<td>2.86 ± 0.94</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>6.57 ± 2.05</td>
<td>4.25 ± 0.50</td>
</tr>
</tbody>
</table>

\( a, b, c, d, e, f \) P<0.05; § harmonic mean ± pseudo SE; AUC(0–∞) = area under serum concentration-time curve; t\(_{1/2,1}\) = distribution half-time; t\(_{1/2,2}\) = terminal half-time; t\(_{1/2,3}\) = absorption half-time; t\(_{1/2,4}\) = elimination half-time; C\(_0\) = serum concentration at time 0; V\(_c\) = volume of distribution in central compartment; Cl\(_B\) = serum clearance; AUC after t\(_{last}\) = percentage of AUC extrapolated to infinity after last sampling time; MRT(0–∞) = mean residence time; V\(_dss\) = volume of distribution at steady state; MAT = Mean absorption time; F = bioavailability; Tmax = observed time for C\(_{max}\).
0.14 µg/ml).

Nimesulide and the 4-APMS metabolite were always detected in urine samples from Group B animals, given 1 mg/kg bwt orally, while the 4-HXN metabolite was detected only in trace amounts. From 2 h post administration, 4-APMS was recovered at higher concentrations than nimesulide in all samples (Table 1). Urine was collected after spontaneous urination only; samplings were not uniform across individuals. It was not possible to collect the total urine output.

**Protein binding**

Nimesulide (94.54 ± 1.25%) and 4-HXN (90.40 ± 2.26%) were bound strongly to serum proteins, while 4-APMS binding was considerably lower (45.00 ± 4.42%).

**Pharmacokinetic analyses**

The time course of nimesulide serum concentrations after i.v. administration was best described by a 2-compartment open model in all animals. A one-compartment open model best fitted the data after oral administration in Groups A and B. Data from Group C were best described by noncompartmental analysis. To compare results in the fasting and nonfasting states, serum concentration data in Group B and orally treated Group A horses were also analysed by the noncompartmental model used in Group C.

Table 2 shows pharmacokinetic parameters obtained by compartmental analyses after i.v. and oral administration at 1.5 mg/kg bwt and after oral administration at 1.0 mg/kg bwt. Table 3 compares pharmacokinetic parameters obtained by noncompartmental analyses in fasting and nonfasting states, following administration of nimesulide at 1.0 mg/kg bwt.

**Discussion**

The pharmacokinetics of nimesulide in the horse is similar to that in the dog (Toutain et al. 2001a). Specifically, after i.v. administration nimesulide distributed slowly and slightly (t1/2α = 0.73 ± 1.07 h, Vdα = 122.12 ± 6.69 ml/kg bwt and Vc = 112.11 ± 1.30 ml/kg bwt) indicating it was probably mainly confined to extracellular fluid. This finding is probably attributable to its extensive protein binding (94.54 ± 1.25%) – a characteristic of NSAIDs in general – and partly to nimesulide’s weak acidity (pKa = 6.56) (Singh et al. 1999), which limits its passage into cells. Nimesulide was eliminated (t1/2β = 6.32 ± 0.91 h; Clβ = 13.71 ± 2.06 ml/h/kg bwt) at similar or slower rates than some other NSAIDs in the horse (elimination half lives: phenylbutazone 3–8 h; naproxen 4–5 h; flunixin 1.6 h; meclofenamic acid 1 h) (Lees and Higgins 1985). According to Lees et al. (2004a), between species differences in NSAIDs clearance and terminal half-life are due mainly to differences in hepatic clearance, since extensive plasma protein binding limits glomerular ultrafiltration and hence renal clearance. In man, nimesulide is almost entirely eliminated by hepatic biotransformation, with negligible amounts of the parent drug eliminated in urine. The elimination half-life and clearance after oral administration in man (t1/2β, 1.96–4.75 h; Clβ 35.2–90.9 ml/h/kg bwt, Bernareggi 1993) appear faster than in the horse, suggesting that metabolic transformation is slower as an elimination route in the latter species. Furthermore, hepatic clearance in the horse was close to body clearance (0.23 ml/min/kg bwt) while liver blood flow is around 24 ml/min/kg bwt (Dyke et al. 1998), so hepatic extraction is very low (E about 0.01), and lower than in man (E = 0.1) (Bernareggi 1993), again indicating that hepatic transformation is slower in the horse than man.

The 4-APMS metabolite was the major nimesulide-related component of horse urine, with levels over 3 times higher than nimesulide itself, as expected from the greater polarity and the lower plasma protein binding (45 ± 4.42%) of this metabolite compared to both nimesulide (94.54 ± 1.25%) and 4-HXN (90.40 ± 2.26%). In man, 5 active (anti-inflammatory and analgesic) metabolites with lower potency than the parent drug, have been identified. The main ones are 4-HXN, present in plasma and urine, and 2-(4’-hydroxyphenoxy)-4-N-acetylamino-methanesulfonanilide, found in urine and faeces; the others, including 4-APMS are present mainly in faeces, but also urine (Bernareggi 1998). Therefore, although the investigation of nimesulide biotransformation was incomplete, reduction (to 4-APMS) appears to be the main metabolic conversion in the horse, in contrast to man where hydroxylation to 4-HXN is the main transformation (Bernareggi 1998). Our findings on 4-APMS in horse urine support the use of this compound as marker for detecting illicit nimesulide use in racehorses (Sarkar et al. 1997).

The bioavailability of nimesulide observed after the oral administration of 1.5 mg/kg bwt in fasted horses (54 ± 8%) was fairly similar to that in fasted dogs administered 5 mg/kg bwt (47 ± 12%) (Toutain et al. 2001a), while absorption was much slower in horse than in dog (MAT horse 6.57 ± 2.88 vs. MAT dog close to 0) (Toutain and Bousquet-Melou 2004). After the 2 different oral doses, the significant (P<0.05) reductions in Cmax and AUC after 1 mg/kg bwt vs. 1.5 mg/kg bwt were dose-related suggesting bioavailability is unlikely to be affected.

Although data on absolute bioavailability in man have not been published, from Bernareggi (1993) one may infer that nimesulide absorption is almost complete after oral administration and is not influenced by formulation (tablet, suspension, granules); furthermore absorption is little influenced by food intake – as is also the case in the dog (Toutain et al. 2001a). By contrast, in the horse we found that the nonfasted state resulted in variably delayed and incomplete absorption and consequently reduced bioavailability. Comparison of AUCs(0–∞) (fasted: 34.06 ± 7.02 h µg/ml; fed: 13.98 ± 4.56 h µg/ml) and MRTs (fasted: 13.61 ± 3.18 h; fed: 12.22 ± 3.28 h) indicated that nimesulide bioavailability in fed horses was about half that of fasted horses. Previous in vitro and in vivo investigations (Gerring et al. 1981; Maitho et al. 1986; Landoni and Lees 1995; Lees et al. 2004a)

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**TABLE 3: Pharmacokinetic parameters (mean ± s.d.) after oral administration in fasted and not fasted horses (noncompartmental analysis) Groups B and C**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 4, fasted)</td>
<td>(n = 7, fed)</td>
</tr>
<tr>
<td>AUC(0–∞) (h.µg/ml)</td>
<td>34.06 ± 7.02a</td>
<td>13.98 ± 4.56a</td>
</tr>
<tr>
<td>AUC after last (%)</td>
<td>16.73 ± 7.46</td>
<td>18.97 ± 11.14</td>
</tr>
<tr>
<td>λ1 (h)</td>
<td>1.23 ± 0.91</td>
<td>0.86 ± 0.50</td>
</tr>
<tr>
<td>λ2 (h)</td>
<td>6.32 ± 0.91</td>
<td>12.22 ± 3.28</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>6.32 ± 0.91</td>
<td>12.22 ± 3.28</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>2.86 ± 0.94b</td>
<td>1.19 ± 0.20b</td>
</tr>
<tr>
<td>Cmax/kg bwt</td>
<td>5.78 ± 1.82c</td>
<td>2.50 ± 0.73c</td>
</tr>
<tr>
<td>MRT(0–∞) (h)</td>
<td>13.61 ± 3.18</td>
<td>12.22 ± 3.28</td>
</tr>
</tbody>
</table>

* a, b, c P<0.05; AUC(0–∞) = area under serum concentration-time curve; AUC after last = percentage of AUC extrapolated to infinity after last sampling time; MRT(0–∞) = mean residence time; Tmax = observed time for Cmax.
have shown clearly that food influences the absorption of various NSAIDs (phenylbutazone, ketoprofen, melcofenamic acid, and flunixin); in particular bioavailability, $C_{\text{max}}$ and $T_{\text{max}}$ in the horse may all be influenced by drug binding to hay and digesta.

The activity of nimesulide against horse COX-1 and COX-2 has been investigated previously by Belloli et al. (2003) using a whole blood assay that takes account of drug binding to plasma proteins, and supplies IC$_{50}$ values more suitable for comparison with plasma concentrations derived from in vivo studies (Pairet and van Ryn 1998; Blain et al. 2002). Nimesulide was found to be preferential for horse COX-2 (IC$_{50}$ COX-1/IC$_{50}$ COX-2 = 12.17), in agreement with findings in the dog (IC$_{50}$ COX-1/IC$_{50}$ COX-2 = 13) (Toutain et al. 2001a). However, concentrations inhibiting both COX-1 (IC$_{50}$ 6.3 µg/ml) and COX-2 (IC$_{50}$ 0.49 µg/ml) were about 7 times higher in the dog than the horse (COX-1 0.86 µg/ml, COX-2 0.07 µg/ml).

High potency against equine cyclooxygenases, compared to the human and canine enzymes (Cryer and Feldman 1998; Pairet and van Ryn 1998; Brideau et al. 2001; Toutain et al. 2001a), has been described for several other NSAIDs (Belloli et al. 2003) and would explain the lower doses required to obtain therapeutic effects (Lees et al. 1991).

Assuming low binding of nimesulide to erythrocytes (Bree et al. 1993), blood IC$_{50}$ can be converted into plasma IC$_{50}$ which are more representative of the in vivo situation. The PCV in the horse is about 0.38, and plasma IC$_{50}$ for nimesulide would therefore be about 1.4 µg/ml for COX-1 and 0.1 µg/ml for COX-2.

Using PK/PD approach Toutain et al. (2001b) showed that in vivo the plasma EC$_{50}$ for nimesulide for antipyretic effect was significantly higher than the IC$_{50}$ calculated in vitro for COX-2 inhibition, while the plasma EC$_{50}$ for anti-inflammatory effect was close to the IC$_{50}$ for COX-1 inhibition; the plasma IC$_{50}$ calculated for COX-1 is therefore probably a good estimate of the in vivo anti-COX-1 activity of nimesulide. Taking this estimate, and considering the daily plasma clearance of 336 ml/kg bwt/day, estimated in the present study, the daily doses of nimesulide required to maintain steady state concentrations (product of daily clearance and IC$_{50}$) at IC$_{50}$ plasma levels for COX-1 and COX-2 in the horse can be estimated at about 0.47 mg/kg bwt and 0.04 mg/kg bwt, respectively. As the oral bioavailability of nimesulide in the horse is about 50% in fasted animals and about 25% in fed horses, these doses have to be increased to about 1.0 and 0.1 mg/kg bwt in fasted and 2.0 and 0.2 mg/kg bwt in fed horses, respectively.

Toutain et al. (2001b) suggested previously that a plasma concentration of 1 µg/ml, near to the plasma IC$_{50}$ for equine COX-1, would produce adequate anti-inflammatory effects, but would be low enough to minimise side-effects. After oral administration of 1 mg/kg bwt to fasted horses in the present study, the plasma concentration of nimesulide exceeded 1 µg/ml for about 10–12 h ($C_{\text{max}}$ = 2.86 ± 0.94 µg/ml), while in fed horses the drug concentration over this level was only 4 of the 7 animals and between 6 and 10 h after treatment. After giving 1.5 mg/kg bwt orally, mean plasma concentration was the range 2.7–0.8 µg/ml from 40 min–24 h after dosing in fasting animals. Based on these findings and considering that several mechanisms in addition to COX inhibition may contribute to the anti-inflammatory action of nimesulide, we suggest that a dose lower than 1.5 mg/kg bwt would be sufficient for antipyretic activity and mild inflammatory conditions; and that 1.5 mg/kg bwt would produce satisfactory anti-inflammatory effects in both fasted and fed animals.

The elimination half-life of nimesulide in the horse (about 7 h) indicates that steady-state concentration is reached in about 2 days (5 times elimination half-life). When administered at a dose in the range 1–1.5 mg/kg bwt there is no substantial accumulation when the drug is given at 12–24 h intervals. The predicted accumulation factor is 1.2 and 1.1 for dosing intervals of 12 and 24 h respectively, favourable values for NSAIDs, which are characterised by limited tolerance, and indicating that a 12–24 h dosing interval is appropriate, depending on the severity of the inflammatory condition.

To conclude, the data obtained for nimesulide provide useful information for the rational and safe use of the drug in horses, in serious and mild inflammatory conditions. In particular, they suggest that the dose required to obtain adequate clinical effects is of the order of 1.5 mg/kg bwt and the dosing interval should be 12–24 h depending on condition severity. However, at this dose, the concentration in the animal exceeds the in vitro IC$_{50}$ for both isoforms, COX-1/COX-2 selectivity is therefore lost and side-effects due to COX-1 inhibition are a possibility. Nimesulide should, therefore, be used with caution in equine clinical practice.

Manufacturers’ addresses

1Sigma-Aldrich, Milan, Italy.
2Gelman Sciences, Ann Arbor, Michigan, USA.
3Roche, Milan, Italy.
4Terumo Europe, Leuven, Belgium.
5Macherey-Nagel, Düren, Germany.
6Perkin Elmer, Monza, Italy.
7Millipore, Milan, Italy.
8Pharsight Corporation, Mountain View, California, USA.
9GraphPad Software, San Diego, California, USA.

Acknowledgements

We thank Helsinn, Lugano, Switzerland, for the support provided, and Don Ward for help with the English. This work was partially supported by funds from the Italian Ministry for University and Scientific and Technological Research (FIRB – 2000).

References


**Author contributions** The initiation, conception and planning of this study were by R.V., C.B., E.F. and S.C. Its execution was by R.V., P.C., C.B., A.Z. and E.F., statistics by R.V., P.C., C.B. and A.Z., and writing by R.V., P.C., C.B. and S.C.