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Naproxen in the horse: Pharmacokinetics and side effects in the elderly

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ABSTRACT

It is well-known that old animals show physiologic and/or pathologic variation that could modify the pharmacokinetics of drugs and the related pharmacodynamic response. In order to define the most appropriate therapeutic protocol in old horses, pharmacokinetic profile and safety of naproxen were investigated in horses aged over 18 years after oral administration for 5 days at the dose of 10 mg/kg b.w./day. After the first administration, the maximum concentration (C_{max} 44.21 ± 9.21 μg/mL) was reached at 2.5 ± 0.58 h post-treatment, the harmonic mean terminal half-life was 6.96 ± 1.73 h, AUC_{0-24h} was 459.71 ± 69.95 h μg/mL, MRT was 7.44 ± 0.74 h and protein binding was 98.47 ± 2.72%. No drug accumulation occurred with repeated administrations. No clinical and laboratory changes were detected after administration of naproxen. Gastric endoscopies performed after the treatment did not show pathological changes of the gastric mucosa.

1. Introduction

Non steroidal anti-inflammatory drugs (NSAID) have been used in equine medicine for over a century (Lees and Higgins, 1985), mainly to treat osteoarticular diseases (Clark and Clark, 1999; Keegan et al., 2008; Schleining et al., 2008; Orsini et al., 2012; Soma et al., 2012) and to relieve abdominal pain (Malone and Graham, 2002; Moses and Bertone, 2002). Their use has also been extended to the treatment of perioperative pain (Prügner et al., 1991; Johnson et al., 1993; Raekallio et al., 1997; Baller and Hendrickson, 2002). NSAIDs side effects are characterized by gastrointestinal, renal and vascular toxicity. In addition, some molecules seem to be able to inhibit the synthesis of cartilage and accelerate the destruction of osteoarthritic joints (Read, 1983; MacAllister et al., 1993; Goodrich et al., 1998; Hough et al., 1999; Caron, 2000; Moses and Bertone, 2002; Monreal et al., 2004).

Naproxen is a NSAID belonging to the group of propionic acid derivatives (Lees and Higgins, 1985; Soma et al., 1995) and acts through a classic mechanism of COX inhibition and consequent block of prostaglandin synthesis. A COX inhibition study in horses have demonstrated its non-selective activity, as in human (Cuniberti et al., 2012). Naproxen is registered for horse use in U.S.A., whereas in Europe it is not licensed, but it is frequently found in urine of racehorses during routine checks (Cagnardi et al., 2011), indicating for a wide off-label use. In horse, naproxen is used for the treatment of soft tissues injuries and for pain and inflammation associated with myopathy (i.e., Tying up) (Lees and Higgins, 1985; Tobin, 1989). In equine experimentally induced myositis, the anti-inflammatory activity of naproxen was found to be superior to that of phenylbutazone (Malone and Graham, 2002; Goodrich and Nixon, 2006; Van Weeren and de Grauw, 2010). After oral administration naproxen has a favorable kinetic profile and a high bioavailability, thus the oral route can be an advantageous route of administration (Cagnardi et al., 2011). Naproxen seems to have a wide safety margin in the horse. In fact, following its oral administration for 6 weeks at doses about 3 times higher than those recommended, no signs of toxicity were observed (Lees and Higgins, 1985; Goodrich and Nixon, 2006). In addition, *in vitro* studies carried out by Bassleer et al. (1992), in human indicated that naproxen does not seem to have deleterious effects on articular chondrocytes.

In human, old patients show adverse reactions to drugs 2–3 times more frequently than younger people (Turnheim, 2004). In fact, as a result of alteration of physiological functions and body composition, changes of pharmacokinetic parameters – i.e., volume of distribution, liver and renal clearance – can be detected (Turnheim, 2003, 2004; Noble, 2003; Cusack, 2004; Klotz, 2009). Such a situation often complicates therapy in this patient's category. In particular, it has been observed that in old human patients naproxen binding to plasma proteins is approximately the half of that observed in younger subjects. Moreover, clearance of the free drug is reduced by a 50%. This suggests the need to reduce the dose in elderly (Upton et al., 1984; McVerry et al., 1986; Gøtzche et al., 1988; Van den Ouweland et al., 1988; Butler and Begg, 2008).

In horses, poor data concerning the pharmacokinetics of naproxen are available (Soma et al., 1995; Cagnardi et al., 2011), and none report any kinetic or tolerability study in old horses.

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Thus, the aim of this study was to evaluate naproxen pharmacokinetic behavior and safety after oral administration in horses aged over 18 years at the dose of 10 mg/kg for 5 days, in order to define the most appropriate therapeutic protocols in old subjects.

2. Materials and methods

2.1. Animals

The study was conducted after favorable opinion of Bioethical Committee of the University of Perugia and in accordance with the EC Council Directive 86/609 EEC (Council of the European Communities, 1986) adopted by the Italian Laws (Decreto Legislativo n. 116, 1992).

Five Haflinger mares aged between 19 and 26 years and weighing 300–450 kg were included in the study. Horses, who usually lived at a semi-wild state, were transferred in a stable and allocated in single stations where they remained for all the experiment's duration. During this period, horses were fed hay twice a day and disposed of *ad libitum* water.

One month prior to the trial, all horses were treated with ivermectin (Equalan®, 200 μg/kg). A coprexamination was performed using the floating method before the beginning of treatments.

2.2. Naproxen treatment and sampling

After 1 week of acclimation, 10 mg/kg b.w./day of naproxen were administered *per os* for 5 consecutive days to each horse. An oral formulation for human use (Naprosyn 250 bag of granules for oral suspension – Recordati SPA, Milano, containing 250 mg of naproxen) appropriately mixed to a small quantity of slightly moist oats was used. Horses were monitored for the duration of the consumption, in order to check that the whole medicated ration was ingested.

Blood samples were taken before the administration of the drug and at fixed experimental time points (0.5, 1, 1.5, 2, 3, 4, 6, 9, 12 and 24 h after the first treatment and 3, 6, 9, 12 and 24 h after the following administrations), from the jugular vein for the pharmacokinetic study. During the first day of sampling, a venous catheter (14 gauge, 7 cm) was placed in order to reduce sampling pain and stress. Blood was immediately centrifuged (3000*g* for 10 min) and the obtained serum was maintained at −20 °C until the analyses.

2.3. Clinical and endoscopic examination

In order to exclude any preexistent pathology precluding drug treatment and to reveal eventual physical, hematological and biochemical changes due to naproxen treatment, horses were subjected, before the drug treatment and then at 24 and 96 h and 20 days after the last treatment, to physical examination and complete blood work (emochromocitometric analysis and biochemical profile, with particular attention to the hepatic and renal function). Blood was analyzed with a Coulter counter (HeCo Vet S, SEAC Radim, Firenze, Italy) and an automatic analyzer (Hitachi 704, Boehringer Manheim, Milano, Italy) respectively for emochromocitometric analysis and biochemical profile. The differential count of leukocytes was manually performed on blood smears.

Furthermore, after sedation with 20 μg/kg of detomidine (Domosedan®, Pfizer, Italia), a gastric endoscopic examination was performed before the 1st drug administration and then repeated 4 days after the end of treatments, using a video endoscope with probe 250 cm in length and 1 cm in diameter (Pentax VSB-2900, Pentax Italia s.r.l. Milano – Italy). Before the endoscopy, horses were feed and water deprived for 24 and 6 h, respectively in order to allow full gastric emptying (Vatistas et al., 1997).

2.4. Determination of naproxen concentration

Concentrations of naproxen in serum samples were determined by high-performance liquid chromatography (HPLC) following a liquid–liquid extraction, slightly modifying the analytical method described by Suh et al. (1995) as reported below.500 μL of serum were added with 1.5 mL of acetonitrile, vortexed and centrifuged at 3500*g* for 10 min. Supernatants were brought at dryness and residues were diluted with 250 μL of mobile phase and injected into the chromatograph (50 μL).

The analytical determination of naproxen was performed by a HPLC Gold System (Beckman, San Ramon, California, USA), equipped with a UV detector set at 254 nm and an analytical column Prodigy 5 μm ODS3 (250 × 4.6 mm) with a guard column C-18 (4 × 3 mm i.d.) (Phenomenex srl; Italy). The mobile phase was KH_2PO_4 0.04 M pH 2.5:acetonitrile (50:50 v:v) and the flow rate stated at 1.2 mL/min.

2.5. Calibration curves

Stock solutions (1 mg/mL) of naproxen was prepared in methanol and stored at −20 °C. Blank serum was fortified with naproxen at different concentrations, in order to obtain a range of 0.5–100 μg/mL. Spiked samples were than processed as previously described.

Calibration curves were obtained by plotting nominal concentrations and obtained naproxen peak areas ratio. Slopes, intercepts and correlation coefficients were determined before each analytical session. Concentrations of naproxen in unknown samples were calculated from the peak area by interpolation of calibration curve.

2.6. Determination of naproxen binding to plasma proteins

Evaluation of drug–protein binding of naproxen was performed *in vitro* on equine drug-free serum. The sera sampled from all experimental horses before administration of naproxen were pooled and the entity of drug binding to plasma proteins was determined by ultrafiltration, at concentrations ranging from 1 to 100 μg/mL. 500 μL of serum were added with each tested concentration, placed in disposable device equipped with a 30 kDa pore size membrane (Amicon, Millipore) and centrifuged at 4500*g* for 30 min. The obtained filtrates were analyzed at the same chromatographic conditions described above.

2.7. Pharmacokinetic and statistical analyses

Pharmacokinetic parameters were calculated from time/concentration curve using a mono-compartmental and non-compartmental model (WinNonLin Prof Version 6.1 – Pharsight Corporation, USA). The model best describing the curve was based on the appearance of the observed and predicted concentrations, reduction in the sums of squares, and application of Akaike's information criterion (MAICE; Yamaoka et al., 1978). All data points were weighted by the inverse squared of the fitted value.

After oral administration data were fitted to the equation:

$$C_{(t)} = \frac{D^* \lambda_1}{V(\lambda_1 - \lambda_2)} * (e^{-\lambda 2t} - e^{\lambda_1 t})$$

where D is the dose, V the volume of distribution, and λ_1 and λ_2 the first order rate constants for the absorption and elimination phases, respectively. Half-times were calculated as $\ln 2/\lambda_n$. The area under the serum concentration–time curve ($AUC_{(0-\infty)}$) and AUC from 0