Pharmacokinetics and synovial fluid concentrations of flurbiprofen enantiomers in horses: chiral inversion

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Flurbirpofen (FBP), a member of the 2-aryl propionate nonsteroidal anti-inflammatory drug class, has potent anti-inflammatory and analgesic properties. The commercial preparation is a racemic mixture of the R(−) and S(+) enantiomers of FBP. In this study, R(−) and S(+) FBP were used to investigate the metabolic chiral inversion. Each enantiomer was administered separately (0.25 mg/kg) and in a racemic mixture (0.5 mg/kg) intravenously to horses. Plasma and synovial concentration of each enantiomer was determined and the disposition of each was analyzed. After intravenous administration of R(−) FBP and S(+) FBP to horses no chiral inversion was detected. After the administration of the FBP racemate and individual enantiomers no differences were observed between pharmacokinetic parameters [$t_{1/2\beta}$ (h), *Cl* (L/h·kg), *AUC* (μg·h/mL), V_{ss} (L/kg) and MRT (h)] for R(−) and S(+) FBF. Synovial fluid concentrations of both FBP enantiomers were lower than plasma concentrations and no stereoselective differences were detected. These data indicate that the disposition of FBF in horses is not enantioselective and demonstrate a difference in the pharmacokinetic behavior of the enantiomers as compared with other 2-aryl-propionic acids, such as carprofen, ketoprofen and vedaprofen in the horse.

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INTRODUCTION

Flurbiprofen (FBF) is a potent 2-aryl-propionic acid anti-inflammatory drug that is prescribed for its anti-inflammatory and analgesic activity. It contains an asymmetric carbon and thus exists in two enantiomeric S(+) and R(−) forms. The S(+) and R(−) enantiomers have different pharmacodynamic potencies (Laneuville *et al.*, 1994; Carabaza *et al.*, 1996). *In vitro* inhibitory effects on cyclo-oxygenase-1 (COX-1) or COX-2 are largely attributable to the S(+) enantiomer, reportedly being 100–10 000 times more potent than the R(−) enantiomer (Peskar *et al.*, 1991; Laneuville *et al.*, 1994; Carabaza *et al.*, 1996). In addition to peripheral action, a central site of action possibly independent of the inhibition of prostaglandin synthesis has been suggested for the anti-nociceptive activity of R(−) FBF in the rat paw formalin test (Geisslinger *et al.*, 1994).

Enantiospecific differences in plasma profiles have been reported after administration of several aryl-2-propinic acid racemates. Differences in pharmacokinetics depend both on the compound and the species (Delatour *et al.*, 1994a,b; Soraci & Benoit, 1996; Soraci *et al.*, 1996a,b; Landoni *et al.*, 1997; Castro *et al.*, 1998, 2000, 2001; Landoni & Soraci, 2001; Igarza *et al.*, 2002, 2004; San Martín *et al.*, 2002). Previous studies have indicated that, after administration of racemates of several 2-aryl-propionic acid drugs to horses, the pharmacokinetic behavior of the enantiomers is different (Jaussaud *et al.*, 1993; Landoni & Lees, 1996; Soraci *et al.*, 1996a,b; Armstrong *et al.*, 1999; Lees *et al.*, 1999). Predominance of the S(+) enantiomer was observed in horses for ketoprofen (KTF) (Jaussaud *et al.*, 1993; Landoni & Lees, 1996; Armstrong *et al.*, 1999) and fenoprofen (FPF) (Soraci *et al.*, 1995a). However, after racemic carprofen (CPF) and vedaprofen administration to horses, the plasma concentration *AUC* ratios R:S were 82:18 and 83:17 respectively (Lees *et al.*, 1991, 1999, 2002). The S(+) enantiomers of KTF and FPF predominate following administration of the racemate due to unidirectional chiral inversion of R (−) to S(+) enantiomers resulting in higher concentration of the S(+) enantiomer in plasma (Jaussaud *et al.*, 1993; Landoni & Lees, 1996; Soraci *et al.*, 1996a,b; Armstrong *et al.*, 1999). When chiral inversion of profens does not occur, other

metabolic stereoselective pathways (aromatic hydroxylation, glucuronidation) may explain the chiral plasma profile of enantiomers (Soraci *et al.*, 1995a). For example, we have shown a strong stereoselective glucuronidation of CPF by the liver in favor of the S(+) enantiomer in horses that explains the higher bioavailability of R(−) CPF enantiomer (Soraci *et al.*, 1995b). Furthermore, studies of the stereoselective penetration of CPF and KTF into equine synovial fluid have been performed (Armstrong *et al.*, 1999; Verde *et al.*, 2001).

Flurbirpofen exhibits stereoselectivity in its pharmacokinetics. Metabolic chiral inversion of R(−) FBP to the S(+) enantiomer and variation between different species has been reported (Menzel-Soglowek *et al.*, 1992; Geisslinger *et al.*, 1994; Davies, 1995; Patel *et al.*, 2003). As there are no reports on stereospecific disposition of FBP for the horse, the goal of this study was to determine the plasma disposition of FBP enantiomers following a single intravenous dose of the racemic mixture and the individual enantiomers, another objective was to determine the FBP enantiomeric profiles in synovial fluid after racemate administration to horses.

MATERIALS AND METHODS

Chiral inversion: animals, drug administration and sampling

Four healthy 7–10 years old mixed breed mares (417 ± 40.3 kg bw) were used in a two-period cross-over study. Animals were housed in individual boxes, fed a maintenance ration twice daily, and allowed free access to water during the study. Animal experiments were approved by the institutional animal welfare committee. Mares were dosed via the jugular vein with a single dosage of 0.25 mg/kg of S(+) FBP and R(−) FBP (PAZ Pharma F &E GmbH, Frankfurt, Germany) (optical purity of each enantiomer of FBP >98 %), prepared in buffer phosphate pH 7.4 and dimethyl sulfoxide (DMSO) (90:10). Blood samples (8 mL) were taken immediately before the injection (control samples) and at postinjection times 5, 10, 15, 30 min and 1, 1.5, 2, 4, 6 and 8 h. The samples were centrifuged at 2000 ***g*** for 10 min and the plasma removed and stored at −20 °C until analysis.

Disposition of racemic FBP in plasma and synovial fluid

Six healthy 8–11 years old mixed breed mares (428 ± 27.4 kg bw) were used. The animals were housed in individual boxes and fed a maintenance ration twice daily, and allowed free access to water during study. Each mare received a single intravenous dose of 0.5 mg/kg racemic flurbiprofen (rac-FBP) (Sigma Chemical Co. St Louis, MO, USA) prepared in buffer and DMSO (90:10). Blood samples (10 mL) were collected by jugular puncture into heparin tubes from each animal at 0, 5,10, 15, 30 min and 1, 1.5, 2, 4, 6 and 8 h. The samples were centrifuged at 2000 ***g*** at 4 °C for 10 min, the plasma removed and stored at −20 °C until analysis.

Synovial fluid was obtained aseptically from left and right intercarpal and radio carpal joint spaces according to the technique of Soraci *et al.* (1996b) at 30 min, 1, 1.5, 2, 4 and 6 h after racemate FBP administration. Synovial fluid was centrifuged at 1500 ***g*** at 4 °C for 5 min and stored at −20 °C until analysis.

Analytical methods and measurements

The plasma samples (500 μL) were acidified with 500 μL of HCl (1 mol/L) and then extracted twice with 8 mL of diethylether. After 20 min of vigorous agitation they were centrifuged at 1000 ***g*** for 5 min and subsequently evaporated to dryness under nitrogen stream at 60 °C.

The dry residue was derivatized with L-leucinamide by a method adapted from Foster and Jamali (1987) (Soraci *et al.*, 1995a). To accomplish the conversion, 100 μL of 50 mmol/L triethylamine (in acetonitrile) (Merk, Darmstadt, Germany), 50 μL of 60 mmol/L ethyl choloroformate (in acetonitrile) (Merk), 50 μL of 1 mol/L L-leucinamide hydrocholoride (in water) (ICN Pharmaceuticals, Costa Mesa, CA, USA), 50 μL of 1 mol/L triethylamine (in methanol) and 50 μL water were successively added to the dry extract. The diasteroisomers so produced were resolved by high-performance liquid chromatography (HPLC). The HPLC gradient system consisted of an LKB (Pharmacia, Bromma, Sweden) pump, model 2249, UV variable detector model 2141 and software HPLC Manager. HPLC separation was carried out under gradient conditions on a Luna® (Phenomenex, Torrance, CA, USA) C18 column (0.4 × 15 cm, 5 μm particle size). The mobile phase was KH_2PO_4 10 mmol/L (A – acetonitrile; B – gradient mixture). At a flow rate of 1.5 mL/min, the elution program started at 35% B followed by a linear gradient to 50% B in 12 min. The mobile phase B was held (50%) for 1 min before it went to the initial condition over 16 min. Detection was by UV at 250 nm. Under these conditions the retention times for R(−) and S(+) FBP were 9.6 and 10.5 min, respectively, with a resolution factor of 3.5. Percentages of recovery of R(−) and S(+)-FBP were 92.80 and 94.61% respectively. The limit of quantification (defined as 10 times as large as the standard deviation of the instrumental noise level, LOQ: 10 × S/N) for the each enantiomer was 0.08 μg/mL. Repeatability was 6%. Calibration curves for both enantiomers were linear over the range of 0.08–10 μg/mL.

Pharmacokinetic parameters were estimated using model-independent methods and fitting the appropriate model to the concentration–time data by means of a PK Solution 2.0® (Summit Research Services, Ashland, OH, USA) computer program. The values reported are mean ± SD. Differences between mean values of enantiomer pharmacokinetic parameters were assessed by *t*-test for paired values ($P > 0.05$). The S(+) FBP and R(−) FBP plasma concentration–time was plotted and the areas under the curve (*AUC*) were measured by the linear trapezoidal rule (Baggot, 1977).The quantification of chiral inversion was determined using the formula of Pang and Kwan (1983) and Beck *et al.* (1991):

$$\%\ \text{Inversion rate} = \frac{AUC_{(S)\ \text{after}\ (R)} \times \text{dose (S)}}{AUC_{(S)\ \text{after}\ (S)} \times \text{dose (R)}} \times 100.$$

RESULTS

The plasma disposition of R(−) FBP after 0.25 mg/kg i.v. administration of R(−) enantiomer is illustrated in Fig. 1. After administration of R(−) FBP the antipode was not detected in plasma. Similarly, the plasma disposition of S(+) FBP after 0.25 mg/kg i.v. administration of S(+) enantiomer is illustrated in Fig. 2. After administration of S(+) FBP the antipode was not detected in plasma.

Plasma concentration curves for R(−) and S(+) FBP following intravenous administration of 0.5 mg/kg racemate are shown in Fig. 3. After the intravenous dosage of 0.5 mg/kg of racemic FBP, the mean plasma enantiomeric proportional R(−):S(+) was 54.6%:45.4% (Fig. 4).

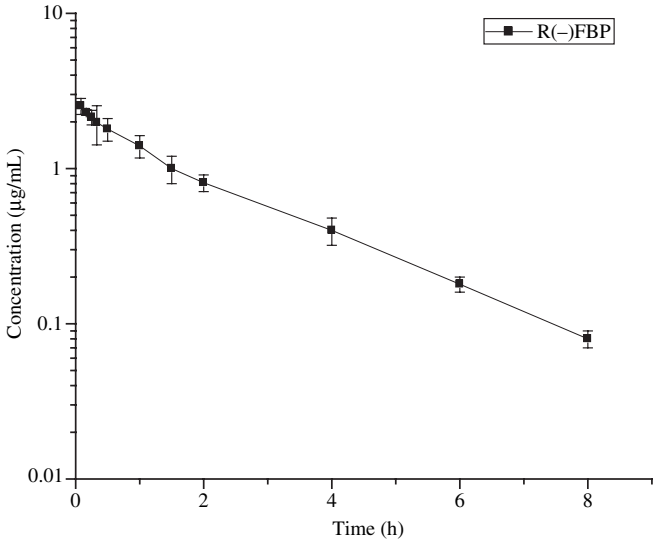

Fig. 1. (R) flurbiprofen plasma concentration after i.v. administration of (R) flurbiprofen at a dosage of 0.25 mg/kg.

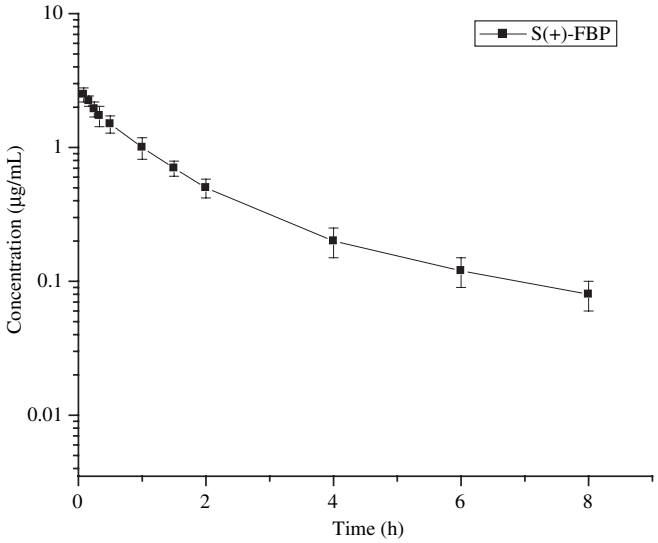

Fig. 2. (S) flurbiprofen plasma concentration after i.v. administration of (S) flurbiprofen at a dosage of 0.25 mg/kg.

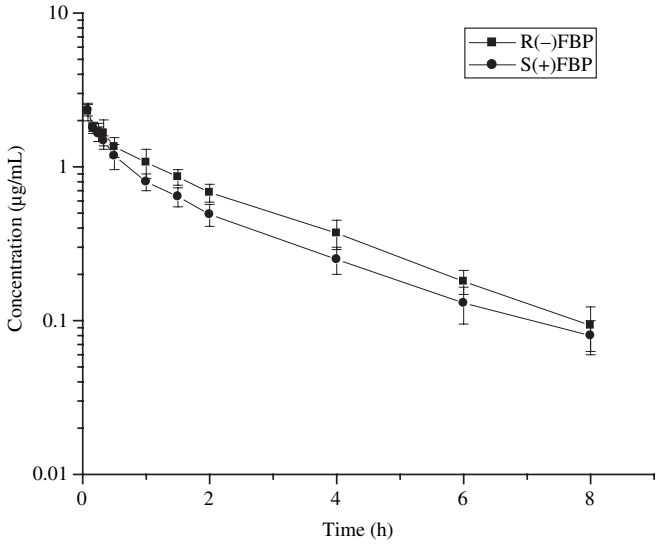

Fig. 3. The (R) and (S) flurbiprofen plasma concentrations after i.v. administration of racemic flurbirpofen at a dosage of 0.5 mg/kg.

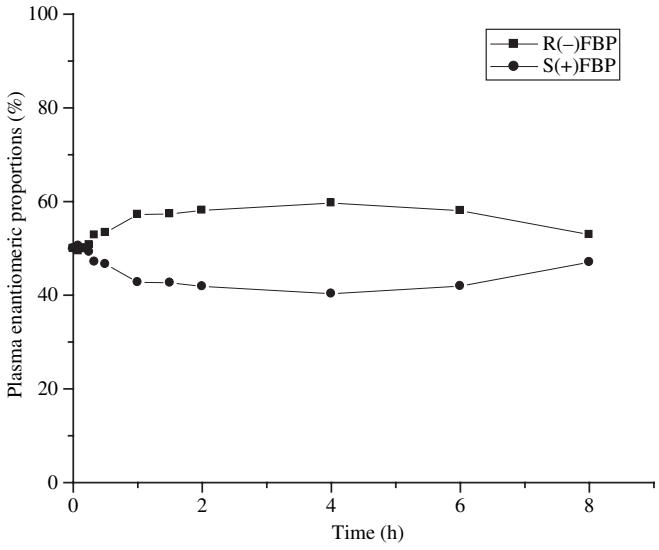

Fig. 4. Plasma enantiomeric proportions (%) vs. time of the (R) and (S) flurbiprofen after i.v. administration of racemic flurbirpofen at a dosage of 0.5 mg/kg.

Pharmacokinetic parameters of (R) and (S) enantiomers of FBP following intravenous administration of the racemate FBP (0.5 mg/kg) and separate FBP enantiomers (0.25 mg/kg) are presented in Table 1. No statistical differences were observed between pharmacokinetic parameters of $t_{1/2\beta}$ (h), *Cl* (L/h·kg), *AUC* (μg·h/mL), V_{ss} (L/kg) and *MRT* (h) for R(−) and S(+) FBF enantiomers. However, the R(−) FBF was predominant in plasma.

The value of area under plasma concentration–time curve (*AUC*) after racemate administration for R(−) FBP was similar to that for S(+) FBP (4.53 ± 0.62 vs. 3.79 ± 0.53). The plasma *AUC*s of R(−) and S(+) FBP after separate administration were 4.90 ± 1.10 and 4.00 ± 0.90 respectively.

Table 1. Some Pharmacokinetic parameters of R(−) and S(+) enantiomers of flurbiprofen following intravenous administration of racemic flurbiprofen (0.5 mg/kg) (a) and separate flurbiprofen enantiomers (0.25 mg/kg) (b) to horses

Parameter	(R) enantiomer	(S) enantiomer
(a) Racemic flurbiprofen		
$t_{1/2\beta}$ (h)	2.08 ± 0.25	1.81 ± 0.13
AUC (μg·h/mL)	4.53 ± 0.62	3.79 ± 0.53
V_{ss} (L/kg)	0.16 ± 0.03	0.20 ± 0.06
Cl (L/h·kg)	0.59 ± 0.10	0.71 ± 0.15
MRT (h)	3.08 ± 0.30	2.64 ± 0.52
(b) Flurbiprofen enantiomers		
$t_{1/2\beta}$(h)	1.90 ± 0.30	1.85 ± 0.22
AUC (μg·h/mL)	4.90 ± 1.10	4.00 ± 0.90
V_{ss} (L/kg)	0.14 ± 0.09	0.18 ± 0.08
Cl (L/h·kg)	0.61 ± 0.18	0.73 ± 0.12
MRT (h)	2.98 ± 0.50	2.59 ± 0.62

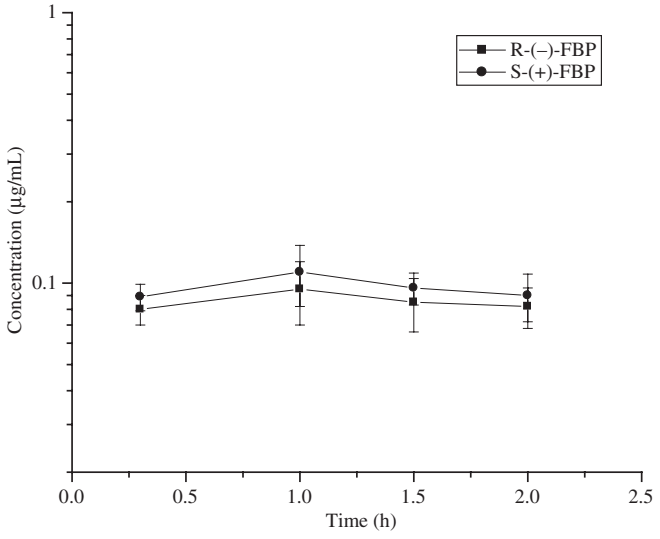

Fig. 5. The (R) and (S) flurbiprofen synovial fluid concentrations after i.v. administration of racemic flurbirpofen at a dosage of 0.5 mg/kg.

Concentrations of the enantiomers in synovial fluid were low, the S(+) enantiomer being more abundant than the R(−) enantiomer (Fig. 5). However, concentrations were monitored for only 2 h. The concentration maximal (C_{max}) for S(+) enantiomer was slightly greater than that of its antipode [0.11 ± 0.028 for (S) and 0.09 ± 0.025 for (R) enantiomer]. Nevertheless, t_{max} values were the same for both enantiomers in the horses treated with racemic FBP. The pharmacokinetic analysis of the synovial fluid was not performed because of the low number of time points at which FBP was detectable.

DISCUSSION

The principal stereochemical mechanisms likely to be involved in the disposition of aryl-propionic anti-inflammatory drugs are metabolic clearance, biliary and renal clearance, and, in this specific chemical series, a unique stereoselective characteristic related to the metabolism, known as chiral inversion (Wechter *et al.*, 1974; Caldwell, 1978; Nakamura *et al.*, 1981; Delatour *et al.*, 1994a,b; Soraci, 1995; Landoni *et al.*, 1997; Landoni & Soraci, 2001). This process comprises the unidirectional inversion of the R(−) enantiomer to its antipode. The molecular mechanism of chiral inversion involves three steps: (i) stereoselective activation of the R(−) profen by formation of an acyl CoA thioester in the presence of co-enzyme A, ATP and Mg^{2+}, (ii) enzymatic epimerization of the R(−) profen thioester to the S(+) thioester; (iii) release of the free, active S(+) profen by hydrolysis of the thioester (Wechter *et al.*, 1974; Nakamura *et al.*, 1981). Chiral inversion has been demonstrated in several organs and tissues (intestine, kidney, lung, muscle, brain and fat) (Cox *et al.*, 1985; Mehvar & Jamali, 1988; Jeffrey *et al.*, 1991; Hall *et al.*, 1992). However, the liver would be the most important one (Berry & Jamali, 1991).

The stereoselective disposition of 2-aryl-propionic acid derivatives in horses has been studied for FPF (Soraci *et al.*, 1996a,b), KTF (Landoni & Lees, 1996; Verde *et al.*, 2001), CPF (Soraci *et al.*, 1995b; Lees, *et al.*, 2002) and vedaprofen (Lees *et al.*, 1999). The results showed large inter-compound variation in enantiomeric disposition behavior in horses. Chiral inversion has been documented in horses for FPF (38%) (Soraci *et al.*, 1996a,b) and KTF (48.8%) (Landoni & Lees, 1996). However, no stereoconversion was reported for CPF (Benoit *et al.*, 1994; Soraci *et al.*, 1995a) and vedaprofen (Lees *et al.*, 1999). Metabolic chiral inversion of R(−) FBP into S(+) FBP and quantitative variation between species has been reported. Indeed, Menzel-Soglowek *et al.* (1992) have determined the fraction inverted in dog, rat, gerbil and guinea-pigs of 39, 2, 5 and 100% respectively. There is negligible R(−) to S(+) inversion in humans (Geisslinger *et al.*, 1994; Davies, 1995). Our results showed that no chiral inversion of R(−) FBP to S(+) FBP occurred after separate intravenous administration of 0.25 mg/kg of R(−) FBP and S(+) FBP in horses. Similarly to R(−) CPF, R(−) FBP does not appear to be a substrate for acyl-coenzyme A ligase (the first metabolic reaction in the chiral inversion). Therefore, these experimental results indicate that in horses, the greater concentration of R(−) FBP in plasma relative to that of S(+) FBP after administration of the racemate is not the consequence of chiral inversion.

The pharmacokinetic results obtained indicate that the disposition of FBF racemate in horses is not enantioselective and demonstrate an important difference in the enantiomeric pharmacokinetic behavior as compared with other 2-aryl-propionic acids, such as CPF, FPF, KTF and vedaprofen (Soraci *et al.*, 1995b, 1996a,b; Landoni *et al.*, 1996). Both enantiomers of FBP possess short elimination half-lives [R(−) FBP: 2.08 h and S(+) FBP: 1.81 h] similar to results reported in horses by Armstrong *et al.* (1999) for R(−) and S(+) KTF (1.89 and 1.00 h respectively). CPF enantiomers are eliminated much more slowly; $t_{1/2\beta}$ values are 18.36 and 9.68 h at a dosage of 0.7 mg/kg for R(−) and S(+) CPF respectively (Soraci *et al.*, 1995b).

The present study confirms that both enantiomers of FBP penetrate rapidly into the synovial fluid, but only at low

concentrations. This rapid distribution may be due to the small unbound available fraction of the drug, and the low concentrations to a high plasma protein binding limiting the extent of transfer of the drug through the synovial membrane in the normal joint (Armstrong *et al.*, 1999). Similar behavior has been described for KTF in horses (Armstrong *et al.*, 1999; Verde *et al.*, 2001). However, these concentrations may increase in swollen joints. Thus, Owens *et al.* (1994) have reported a substantial increase in concentrations for KTF in model equine synovitis (6.5 times relative to normal joints). FBP also attained good concentrations in synovial fluid of human patients (Davies, 1995). In the present study, no significant difference was observed between enantiomers of FBP in synovial fluid. Inversely to the plasma enantiomeric profile, S(+) FBP attained marginally higher concentrations that R(−) FBP in synovial fluid. Concentrations in synovial fluid are at or only slightly above of the limit of quantification. Although no significant difference was found in the volume of distribution between enantiomers of FBP, the slight predominance of the S(+) enantiomer might have been due to a greater volume of distribution of this enantiomer. Low concentrations of enantiomers for a short period of time have been reported in synovial fluid after administration of 2-arylpropionic compounds in horses (Brink *et al.*, 1998; Armstrong *et al.*, 1999; Verde *et al.*, 2001).

Our results demonstrated different inter-compound stereoselective behavior of profens in the same species. The use of racemic compounds vs. active enantiomers has been widely discussed and considered as reasonable from a therapeutic point of view provided that the (R) enantiomer of the racemate undergoes extensive chiral inversion to the S(+) active enantiomer (Delatour *et al.*, 1994a,b). However, for the case of FBP in horses, the clinical use of the racemate could be based on some characteristics: (i) no stereospecific or stereoselective differences between enantiomers, which allows a better handling of the administered dose, (ii) although (S) FBP is the therapeutically relevant anti-inflammatory agent, clinical relevance has been demonstrated for the (R) FBP enantiomer as analgesic in humans.

A central site of action independent of prostaglandin synthesis inhibition has been reported to the anti-nociceptive activity following systemic administration of (R) FBP. Finally, Kulmacz and Lands (1985) have found that R FBP does not inhibit COX1, lowering the potential toxic effects derived from the inhibition of this enzyme. These pharmacological properties make the FBP racemate a potential therapeutic agent in horses.

REFERENCES

Armstrong, S., Tricklebank, A., Lake, S. & Lees, P. (1999) Pharmacokinetics of carprofen enantiomers in equine plasma and synovial fluid – a comparison with ketoprofen. *Journal of Veterinary Pharmacology and Therapeutics*, **22,** 196–201.

Baggot, D. (1977) Principles of drug disposition in domestic animals. In *The Basics of Veterinary Clinical Pharmacology*. Ed. Baggot, D. pp. 1–22. W.B. Saunders, Philadelphia, PA.

Beck, W., Geisslinger, G., Engler, H. & Brune, K. (1991) Pharmacokinetics of iburpofen enantiomers in dogs. *Chirality*, **3,** 165–169.

Benoit, E., Soraci, A. & Delatour, P. (1994) Chiral inversion as a parameter for interespecies and intercompound discrepancies in enantiospecifc pharmacokinetics. *6th International Congress of the European Association of Veterinary Pharmacology and Therapeutics*, 7–11 August, Edinburgh, UK. pp. 153–154.

Berry, B.W. & Jamali, F. (1991) Presystemic and systemic chiral inversion of (R)-(−) fenoprofen in the rat. *Journal of Pharmacology and Experimental Therapeutics*, **258,** 695–701.

Brink, P., DeGraves, F., Ravis, W., Johansen, D., Campbell, J. & Duran, S. (1998) Stereospecific pharmacokinetics of free and protein bound ketoprofen in serum and synovial fluid of horses after intravenous and intramuscular administration. *American Journal of Veterinary Research*, **59,** 739–743.

Caldwell, J. (1978) Structure-metabolism relationships in amino acid conjugation. In *Conjugation Reactions in Drug Biotransformation*. Ed. Aitio, A. pp. 11–112. Elsevier/North Holland, Amsterdam.

Carabaza, A., Cabré, F., Rotlan, E., Gómez, M., Gutiérrez, M., García, L. & Mauleón, D. (1996) Stereoselective inhibition of inducible cyclooxygenase by chiral nonsteroidal anti-inflammatory drugs. *Journal of Clinical Pharmacology*, **36,** 505–512.

Castro, E., Soraci, A., Tapia, O. & Fogel, F. (1998) A preliminary study of the pharmacokinetics of fenoprofen enantiomers following intravenous administration of the racemate to cats. *Veterinary Research Communications*, **22,** 1–6.

Castro, E., Tapia, O., Fogel, F. & Soraci, A.L. (2000) Chiral inversion of R-(−) fenoprofen and ketoprofen enantiomers in cats. *Journal of Veterinary Pharmacology and Therapeutics*, **23,** 265–271.

Castro, E.F., Soraci, A.L., Fogel, F., Franci, R. & Tapia, O. (2001) Disposition of suprofen enantiomers in the cat. *The Veterinary Journal*, **162,** 38–43.

Cox, J.W., Cox, S.R., Van Giessen, G. & Ruwart, M.J. (1985) Ibuprofen stereoisomer hepatic clearance in normal and fatty *in situ* perfused rat liver. *Journal of Pharmacology and Experimental Therapeutics*, **232,** 1984–1985.

Davies, N.M. (1995) Clinical pharmacokinetics of flurbiprofen and its enantiomers. *Clinical Pharmacokinetics*, **28,** 100–114.

Delatour, P., Benoit, E., Besse, S. & Soraci, A. (1994a) Asymétrie moléculaire et pharmacologie comparée. *Revue de Médicine Vétérinaire*, **145,** 551–561.

Delatour, P., Benoit, E. & Soraci, A. (1994b) Drug chirality: its significance in veterinary pharmacology and therapeutics. *6th International Congress of the European Association of Veterinary Pharmacology and Therapeutics*, 7–11 August, Edinburgh, UK. pp. 6–9.

Foster, R.T. & Jamali, F. (1987) High performance chromatographic assay of ketoprofen enantiomers in human plasma and urine. *Journal of Chromatography*, **416,** 338–393.

Geisslinger, G., Ferreira, S.H., Menzel, S., Schlott, D. & Brune, K. (1994) Antinociceptive actions of R(−)-flurbiprofen a non cyclooxygenase inhibiting 2-arylpropionic acid in rats. *Life Science*, **54,** PL173–PL177.

Hall, S.D., Hassanzadeh-Khayyat, M., Knadler, M.P. & Mayer, P.R. (1992) Pulmonary inversion of 2-arylpropionic acids: influence of protein binding. *Chirality*, **4,** 349–352.

Igarza, L.M., Soraci, A., Auza, N. & Zeballos, H. (2002) Chiral inversion of (R)-ketoprofen in bovines: influence of age and different physiological states in dairy cattle. *Veterinary Research Communications*, **26,** 29–37.

Igarza, L.M., Soraci, A., Auza, N. & Zeballos, H. (2004) Some pharmacokinetic parameters of R(−) S(+)-ketoprofen: the influence of age and differing physiological status in dairy cattle. *Veterinary Research Communications*, **28,** 81–87.

Jaussaud, P.J., Bellon, S., Besse, D., Courtout, D. & Paul, D. (1993) Enantioselective pharmacokinetics of ketoprofen in horses. *Journal of Veterinary Pharmacology and Therapeutics*, **20,** 166–167.

Jeffrey, P., Tucker, G.T., Bye, A., Crewe, H.K. & Wright, P.A. (1991) The site of inversion of (R)-(−)-ibuprofen: studies using rat *in situ* perfused rat liver. *Journal of Pharmacy and Pharmacology*, **43,** 715–720.

Kulmacz, R.J. & Lands, W.E.M. (1985) Stoichiometry and kinetics of the interaction of prostaglandin H synthase with anti-ínflammatory agents. *Journal of Biological Chemistry*, **260,** 12572–12578.

Landoni, M.F. & Lees, P. (1996) Pharmacokinetics and pharmacodynamics of ketoprofen enantiomers in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, **19,** 466–476.

Landoni, F. & Soraci, A.L. (2001) Pharmacology of chiral compounds 2-arylpropionic acid derivatives. *Current Drug Metabolism*, **2,** 37–51,

Landoni, M.F., Soraci, A.L., Delatour, P. & Lees, P. (1997) Enantioselective behaviour of drug use in domestic animals. *Journal of Veterinary Pharmacology and Therapeutics*, **20,** 1–16.

Laneuville, O., Breuer, D.K., Dewitt, D.L., Hla, T., Funk, C.D. & Smith, W.L. (1994) Differential inhibition of human prostaglandin endoperoxide H synthase-1 and -2 by nonsteroidal anti-inflammatory drugs. *Journal of Pharmacology and Experimental Therapeutics*, **271,** 927–934.

Lees, P., Delatour, P., Benoit, E. & Foster, A.P. (1991) Pharmacokinetics of carprofen enantiomers in the horse. *Acta Veterinaria Scandinavica*, **87** (Suppl.), 249–251.

Lees, P., May, S.A., Hoeijmakers, M., Coert, A. & Rens, P.V. (1999) A pharmacodynamic and pharmacokinetic study with vedaprofen in an equine model of acute nonimmune inflammation. *Journal of Veterinary Pharmacology and Therapeutics*, **22,** 96–106.

Lees, P., Aliabadi, F.S. & Landoni, M.F. (2002) Pharmacodynamics and enantioselective pharmacokinetics of racemic carprofen in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, **25,** 433–448.

Mehvar, R. & Jamali, F. (1988) Pharmacokinetic analysis of enantiomeric inversion of chiral nonsteroidal anti-inflammatory drugs. *Pharmaceutical Research*, **5,** 76–79.

Menzel-Soglowek, S., Geisslinger, G., Beck, W.S. & Brune, K. (1992) Variability of inversion of (R)-flurbiprofen in different species. *Journal of Pharmaceutical Science*, **81,** 888–891.

Nakamura, Y., Yamaguchi, T., Takahashi, S., Hashimoto, S., Iwatani, K. & Nakagawa, Y. (1981) Optical isomerisation mechanism of (R)-(−)-hydratopic acid derivatives. *Journal of Pharmacobio-Dynamics*, **4,** S-1–S-x.

Owens, J., Kamerling, S. & Keowen, M. (1994) Anti-inflammatory effects and pharmacokinetics of ketoprofen in a model of equine synovitis. In *European Journal Association of Veterinary Pharmacology and Toxicology*. Ed. Lees, P. 6th International Congress, Edinburgh. Blackwell Scientific Publications, Oxford, pp. 170.

Pang, K.S. & Kwan, K.C. (1983) A commentary: methods and assumptions in the kinetic estimation of metabolite formation. *Drug Metabolism and Disposition*, **11,** 79–84.

Patel, B.K., Jackson, S.H., Swift, C.G. & Hutt, A.J. (2003) Disposition of flurbiprofen in man: influence of stereochemistry and age. *Xenobiotica*, **33,** 1043–1057.

Peskar, B.M., Kluge, S., Peskar, B.A., Soglowek, S.M. & Brune, K. (1991) Effects of pure enantiomers of flurbiprofen in comparison to racemic flurbiprofen on eicosanoid release from various rat organs ex vivo. *Prostaglandins*, **42,** 515–531.

San Martín, M.F., Soraci, A., Fogel, F. & Tapia, O. (2002) Chiral inversion of R-(−)-fenoprofen in guinea pigs pretreated with clofibrate. *Veterinary Research Communications*, **26,** 323–332.

Soraci, A.L. (1995) Metabolisation stereoselective compare de acides aryl-2-propioniques: inversion chiral and glucuronoconjugaison. Thèse de Doctorat, Université Claude Bernard Lyon I.

Soraci, A. & Benoit, E. (1996) In vitro fenoprofenyl-coenzyme A thioester formation: interespecies variations. *Chirality*, **7,** 534–540.

Soraci, A.L., Benoit, E. & Delatour, P. (1995a) Comparative metabolism of (−)(R)-fenoprofen in rats and sheep. *Journal of Veterinary Pharmacology and Therapeutics*, **18,** 167–171.

Soraci, A., Benoit, E. & Delatour, P. (1995b) Enantioselective glucuronidation and subsequent biliary excretion of carprofen. *American Journal of Veterinary Research*, **56,** 358–361.

Soraci, A., Jaussaud, P., Benoit, E. & Delatour, P. (1996a) Chiral inversion of fenoprofen in horses and dogs: in vivo-in vitro study. *Veterinary Research*, **27,** 13–22.

Soraci, A.L, Errecalde, J.O. & Mestorino, N. (1996b) Pharmacokinetics of cefoperazone in horse. *Journal of Veterinary Pharmacology and Therapeutics*, **19,** 39–43.

Verde, C.R., Simpson, M.I., Frigoli, A. & Landoni, M.F. (2001) Enantiospecific pharmacokinetics of ketoprofen in plasma and synovial fluid of horses with acute synovitis. *Journal of Veterinary Pharmacology and Therapeutics*, **24,** 179–185.

Wechter, W.J., Loughead, D.G., Reischer, R.J., van Giessen, G.J. & Kaiser, D.G. (1974) Enzymatic inversion of saturated carbon: nature and mechanism of the inversion of R-(−)-*p*-isobutylhidratropic acid. *Biochemical and Biophysical Research Communications*, **61,** 833–837.