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Pharmacokinetic Parameters of (*R*)-(−) and (*S*)-(+)-Flurbiprofen in Dairy Bovines

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ABSTRACT

The aim of the study was to evaluate the pharmacokinetics of flurbiprofen (FBP) in different age groups and physiological status groups in dairy cattle. Ten Argentine Holstein bovines were divided into three different groups: 3 cows in early lactation, 3 cows in gestation and 4 newborn calves. Based on previous experience, all the animals received racemic FBP (50:50) at a dose of 0.5 mg/kg by intravenous administration. Blood samples were taken at predetermined times after administration of flurbiprofen. Plasma enantiomer concentrations were measured by HPLC. Total body clearance (ClB) of (*S*)-(+)-FBP was higher in calves than in cows (114.5, 136.4, 121.4, 128.9 μg/ml vs 22.0, 24.2, 46.5 μg/ml and 27.6, 25.3, 34.6 μg/ml). In calves the disposition kinetics showed stereoselective behaviour. Area under the concentration–time curve (AUC) was higher and Cl_B and steady-state volume of distribution (V_{ss}) were lower for (*R*)-(−)-FBP than for (*S*)-(+)-FBP. In cows, stereoselectivity was observed in Cl_B and elimination half-life ($\frac{t_1}{2}$) only in the early lactation group. In this study, enantioselective metabolic behaviour of FBP under the physiological situations studied was found. Hence, it is possible that both enantiomers of flurbiprofen may contribute to the drug's therapeutic effects, but further studies with the administration of separate enantiomers will be required to elucidate their metabolism.

Keywords: cattle, enantiomer, flurbiprofen, pharmacokinetics

Abbreviations: FBP, flurbiprofen; R-(−) and S-(+), enantiomers of flurbiprofen; AUC, area under curve; $t_{1/2}$, elimination half-life; Cl_B, total body clearance; HPLC, high-performance liquid chromatography; MRT, mean residence time; V_{ss}, volume of distributional at steady state

INTRODUCTION

Flurbiprofen (racemic 2-[2-fluoro-4-biphenyl]propionic acid) is a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of pain or inflammation, in humans. Although it possesses a chiral centre, with the *S*-(+) enantiomer having most of the beneficial activity, both enantiomers may possess analgesic activity and all flurbiprofen preparations to date are marketed as the racemate (Kantor, 1986). The drug was introduced into the North American market in 1986 and has been available internationally since 1977 for humans (Brodgen *et al*., 1979; Davies, 1995). Flurbiprofen exhibits stereoselectivity in its pharmacokinetics. Inversion of (*R*)-(−)-flurbiprofen to its optical antipode occurred to a variable extent in the dog (0.39) and the guinea-pig (1.00) and to a much lower extent in the rat (0.02) and the gerbil (0.05) (Menzel-Soglowek *et al.*, 1992) and it does not appear to undergo enantiomeric inversion in humans (Jamali *et al*., 1988; Geisslinger *et al*., 1994). Administered as a racemic mixture, flurbiprofen is oxidized to

form 4′-hydroxyflurbiprofen and 3′,4′-dihydroxyflurbiprofen, or further methylated to form 3′-hydroxy-4′methoxyflurbiprofen. These metabolites as well as the parent compounds are also subject to conjugation by either glucuronidation or sulphation. With respect to oxidation, the formation of 4′-hydroxyflurbiprofen accounts for 86% of the oxidative metabolites and occurs via the P450 enzyme system in humans (Risdall *et al*., 1978; Szpunar *et al*., 1987; Knadler and Hall, 1989; Tracy *et al*., 1995, 1996).

To date, no studies have been carried out on the enantioselective metabolism of flurbiprofen in bovines. The aim of this work was to study the possible differences in the disposition of flurbiprofen enantiomers that may result from age and different physiological status in dairy cattle following a single intravenous injection of racemic flurbiprofen.

MATERIALS AND METHODS

Animals, drug administration and sampling

Ten clinically normal Argentine Holstein cattle, were divided into three different groups: 3 cows in early lactation (3 days postpartum), 3 cows at 6 months of gestation and at 8 months of lactation; and 4 preruminants (weighting 40 ± 3 kg) of age 3 days. These animals belonged to a farm in production, which limited the number of animals used in the experiment. The cows were fed a high-forage diet and the new-born calves received a milk diet. All animals received racemic flurbiprofen (50:50) at a dose of 0.5 mg/kg of a solution of FBP (Sigma) prepared in phosphate buffer pH 7.4 and DMOS (90:10) by intravenous administration. Blood samples were collected at 5, 10, 15, 30 min and ten at intervals of 30 min until 4 h after dosing in all animals. The samples were collected in heparinized tubes. The plasma was separated by centrifugation at 2000*g* for 5 min and stored at -20°C until analysis.

Analytical methods and measurements

The plasma samples (500 µl) were acidified with 500 µl HCl (1 mol/L) and extracted twice with 8 ml of anhydrous ethyl ether, and subsequently evaporated to dryness under a stream of air at 37°C. The dry residue was derivatized with L-leucinamide by a method adapted from Foster and Jamali (1987) (Soraci *et al*., 1995). The diasteroisomers so produced were analysed by HPLC. The HPLC gradient system consisted of an LKB (Pharmacia, Bromma, Sweeden), pump model 2249, UV variable detector model 2141 and HPLC management software. The compounds were eluted from an RP 18 column (0.4×15 cm, 5 µm particle size) with a mobile phase of acetonitrile–K_2PO_4 10 nmol/L mixture and a gradient from 35% to 50% over 16 min at a flow rate of 1.5 ml/min. Detection was by UV at 250 nm. Under these conditions the retention times for (*R*)-(−) and (*S*)-(+)-flurbiprofen were 10.50 and 11.15 min, respectively, with a resolution factor of 3.5. The absolute recoveries were 92.8% for *R*-(−) and 91.6% for (*S*)-(+)-flurbiprofen. The limit of quantification (defined as ten times the standard deviation of the instrumental noise level) for each enantiomer was 0.25 µg/ml. Repeatability was 6%. Detection was linear ($R = 9.998$) for the two isomers between 0.25 µg/ml and 10 µg/ml.

Data analysis

Pharmacokinetic analysis of plasma disposition curves of (*S*)-(+)- and (*R*)-(−)-flurbiprofen in individual animals was carried out using a non-compartmental method and fitting the concentration–time data to an appropriate model by means of a pK Solution 2.0 computer program (Summit Research Services, Ashland, OH, USA). The area under the curve (AUC) for both (*R*)-(−)- and (*S*)-(+)-flurbiprofen was estimated by the method of trapezoids, and extrapolation to infinity was calculated from C_{last}/β, where C_{last} is the last measured plasma drug concentration (Baggot, 1978). Volume of distribution at steady state (V_{ss}) and body clearance (Cl_B) were calculated by classical methods (Gibaldi and Perrier, 1982).

Statistical analysis

Raw data are presented by categories of animals. In addition each enantiomer was analysed separately. Pharmacokinetic parameters for the different categories of animals and for the different enantiomers of flurbiprofen were statistically compared by applying the Kuskal–Wallis test using the PROC NPAR1WAY procedure of the SAS V8.2 (SAS Institute Inc., Cary, NC, USA). The differences between enantiomers for the calves and both categories of cows were evaluated by Wilcoxon signed rank test (Conover, 1971). Differences were considered to be statically significant at $p < 0.05$.

RESULTS

The plasma concentration–time profiles of flurbiprofen enantiomers and the enantiomeric percentage relation, following intravenous administration of the racemic drug to cows in gestation, to cows in early lactation and to new-born calves are presented in Figures 1, 2 and 3. Pharmacokinetic parameters for *R*-(−) and (*S*)-(+)-flurbiprofen derived from an examination of the individual data are presented in Table I.

Values of Cl_B and V_{ss} were higher and the AUC was lower for (*S*)-(+) than for (*R*)-(−)-flurbiprofen in calves than in cows ($p < 0.05$). There was no statistical difference in pharmacokinetic parameters between the two categories of cows.

In calves, the pharmacokinetics had a stereoselective behaviour. In plasma, (R)-flurbiprofen predominated over (S)-flurbiprofen (AUC 5.70 vs 2.05 (μg min)/ml). V_{ss} was higher for (*S*)-flurbiprofen than for (*R*)-flurbiprofen (338.25 vs 139.10 ml/kg) Cl_B was lower for (R)-flurbiprofen than for (S)-flurbiprofen (49.3 vs 125.3 ml/(minkg)). In cows in early lactation, enantioselectivity was also detected. Significant differences in Cl_B (22.77 vs 29.17 ml/(minkg)) and $t_{1/2}$ (2.76 vs 2.44 min) between (R)- and (S)-flurbiprofen were observed.

DISCUSSION

In this study, V_{ss} was larger in newborn calves than in adult cows because of the larger extracellular fluid volume in the newborn (Reiche, 1983; de Backer, 1986). The continuous

TABLE I
Stereoselective pharmacokinetic parameters for (*R*)-(−)- and (*S*)-(+)-flurbiprofen obtained after intravenous administration of 0.5 mg/kg racemic flurbiprofen in newborn calves, cows in early lactation and cows in gestation

Pharmaco kinetic parameter	Enantiomer	Cows in gestation ($n = 3$)	Cows in early lactation ($n = 3$)	Newborn calves ($n = 4$)	p
AUC ((μg min)/ml)	*R*	11.10, 12.30, 7.10[a]	14.50, 15.0, 7.20[a]	3.10, 5.80, 6.10, 7.80[a]	0.082
	S	11.40, 10.20, 5.40[a]	9.10, 9.90, 7.20[a]	2.30, 1.80, 2.10, 2.00[b*]	0.035
MRT (min)	*R*	3.60, 3.20, 2.20[a]	4.30, 4.60, 3.60[a]	3.00, 3.00, 2.80, 3.20[a]	0.064
	S	3.60, 2.90, 2.20[a]	3.40, 4.60, 3.20[a]	3.30, 3.10, 3.10, 2.90[a]	0.223
Cl_B (ml/(min kg))	*R*	22.50, 20.40, 35.30*a*	17.20, 16.30, 34.80[a]	80.60, 43.50, 40.80 32.30[a]	0.082
	S	22.00, 24.20, 46.50[a]	27.60, 25.30, 34.60[a*]	114.50,136.40,121.40, 128.90[b*]	0.035
$t_{1/2}$ (min)	*R*	2.42, 2.16, 1.57[a]	2.95, 3.05, 2.29 [a]	1.90, 1.96, 1.76, 1.97[a]	0.078
	S	2.75, 1.89, 1.58[a]	2.24, 2.99, 2.10[a*]	2.00, 1.98, 1.86, 1.73[a]	0.147
V_{ss} (ml/kg)	*R*	78.30, 63.30, 79.70[a]	73.3, 77.8, 114.9[a]	221.6, 123.3, 119.0, 92.5[a]	0.095
	S	87.00, 66.60, 105.70[a]	89.3, 109.2, 104.7[ab]	316.1, 389.4, 325.6, 321.9[b]	0.024

AUC, are a under curve; $t_{1/2}$, elimination half-life; Cl_B, total body clearance; MRT, mean residence time; V_{ss}, volume of distribution at steady state
[a,b]Different superscript letters in rows indicate significant differences between animals ($p < 0.05$)
*Significant difference between (*R*)-(−) and (*S*)-(+) enantiomers: ($p < 0.05$)

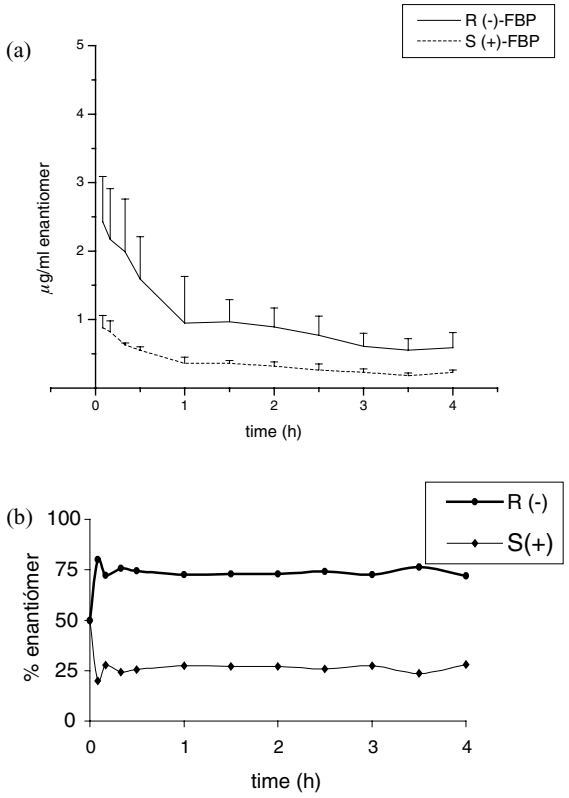

Figure 1. (a) Mean enantiomeric plasma concentrations and (b) enantiomeric percentage relation obtained after intravenous administration of 0.5 mg/kg racemic flurbiprofen in newborn calves ($n = 4$)

changes in physiology and in body composition that occur in newborn animals can alter the distribution pattern of the drug. The most important changes in the body after birth are a decrease in total body water, an increase in adipose tissue, an increase in skeletal mass, and often an increase in plasma protein binding (Baggot, 1994; Sato, 1997). Therefore, a higher volume of distribution at birth should be expected for polar drugs (NSAIDs) (Nouws, 1992). Other investigators showed that the V_{ss} values of flunixin, ketoprofen and phenylbutazone (NSAIDs) are larger in newborn foals than in adult horses (Crisman *et al*., 1996; Wilcke *et al*., 1993, 1998).

The higher Cl_B values in calves could be attributable to differences in extent of distribution, rate of metabolism and/or rate of renal excretion. The overall renal function seems to be mature within 1–2 weeks after birth in ruminant species (de Backer, 1986). Moreover, the enzymatic activities of the different biotransformation reactions do not increase at the same rate throughout life (Shoaf *et al.*, 1987). The flurbiprofen enantiomer is oxidized and then also subjected to conjugation by either glucuronidation or sulphation. Dannenberg and Yang (1992) demonstrated that dietary lipid resulted in a 2-fold and 3-fold increases in the amount

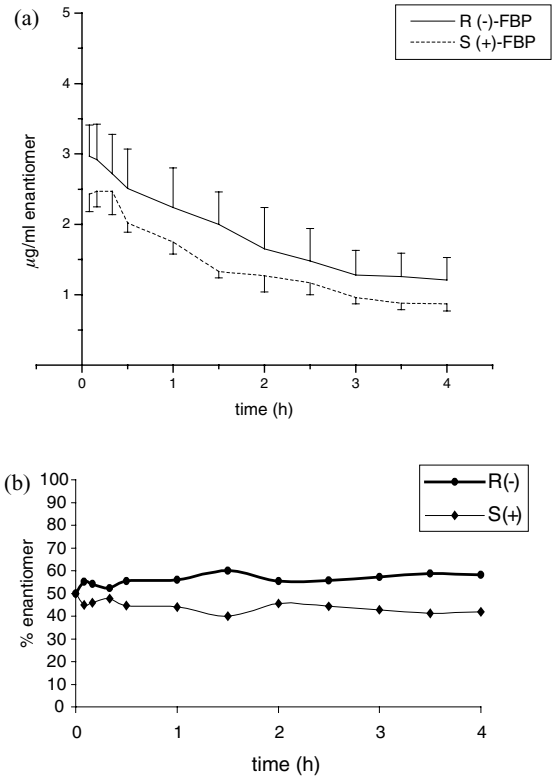

Figure 2. (a) Mean enantiomeric plasma concentrations and (b) enantiomeric percentage relation obtained after intravenous administration of 0.5 mg/kg racemic flurbiprofen in cows in early lactation ($n = 3$)

of UDP-glucuronosyltransferase versus a fat-free diet UDP-glucuronosyltransferase could increase in calves fed with milk, a diet with high lipid content.

The disposition of flurbiprofen in cattle had a stereoselective behaviour in newborn calves. In plasma the (*R*)-(−) enantiomer predominated following administration of racemic flurbiprofen. It had lower V_{ss} and Cl_B values with respect to the (*S*)-(+) enantiomer. This may be attributable to enantioselectivity in plasma protein binding, the rate of metabolism, rate of renal excretion, to chiral inversion of the (*R*)-(−) to the (*S*)-(+) enantiomer or to a combination of these factors. Whether these differences in calves reflect enantiomeric differences in metabolism and/or excretion or whether they are due to chiral inversion of (*R*)-(−) to S(+)-flurbiprofen is not known. However, the latter seems unlikely, since chiral inversion of flurbiprofen has been shown not to occur to a measurable extent in pilot studies carried out by our group (unpublished data) with intravenous administration of (*R*)-(−)- and (*S*)-(+)-flurbiprofen in calves.

The principal urinary metabolites of 2-arylpropionates are acylglucuronides, and marked stereoselectivity in this reaction for (*R*)-(+)-flurbiprofen in humans and rats has been

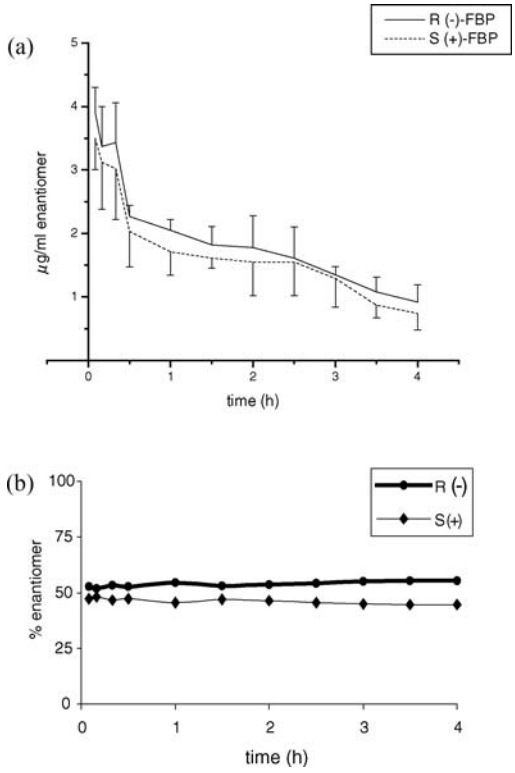

Figure 3. (a) Mean enantiomeric plasma concentrations and (b) enantiomeric percentage relation obtained after intravenous administration of 0.5 mg/kg racemic flurbiprofen cows in gestation ($n = 3$)

demonstrated (Jamali *et al*., 1988). This metabolic pathway seems to be both drug- and species-dependent. For example, the excretion of *S*-(+) conjugates exceeds that of (*R*)-(−) conjugates after administration of benoxaprofen and ibuprofen in man, whereas excretion of the (*R*)-(−) enantiomer is greater than that of (*S*)-(+)-KTP conjugates of fenoprofen and ketoprofen in rabbits (Abas and Meffin, 1987; Caldwell *et al*., 1988). These results might indicate stereoselective hydroxylation and conjugation, and also preferential biliary and urinary excretion of (*S*)-flurbiprofen in calves. Additionally, some biliary recycling of the metabolite and some excretion of unchanged drug in faeces has been suggested in other species (Davies, 1995; Szpunar *et al*., 1987). According to a previously published report, *R*-(−)- and (*S*)-(+)-glucuronides are excreted in bile and subsequently undergo hydrolysis followed by re-absorption of both (*R*)-(−)- and (*S*)-(+)-flurbiprofen, since no intestinal enantioselective hydrolysis has been shown (Eeckhoudt *et al*., 1997). Interspecies comparison revealed that the glucuronidation of flurbiprofen was highly efficient in liver microsomes of humans, monkeys, rats and guinea-pigs, in decreasing order. The (*R*)-(−) enantiomer was glucuronidated more rapidly than the (*S*)-(+) form in liver microsomes of rats and humans (Hamdoune *et al*., 1995). Similar results have been described for other NSAIDs in calves.

Delatour and colleagues (1996) showed small to moderate, but significant, differences in the pharmacokinetics of the (*R*)-(−)- and (*S*)-(+) enantiomers of carprofen in cattle, where the plasma concentrations of the (*R*)-(−) enantiomer exceed those of the (*S*)-(+) enantiomer. Further studies are warranted to clarify the metabolism or flurbiprofen and to determine the extent and possibility of stereoselectivity in biliary excretion of the newborn calves. An enantiomeric difference related to age has been found previously following administration of (*R*)-(−)- and (*S*)-(+)-ketoprofen; the half life in calves was longer than in cows (Igarza *et al*., 2004). These results indicate the metabolic differences in the same species between these compounds of similar chemical structure. The participation of different enzymes in their metabolism might explain the differences.

In cows in early lactation, the disposition of flurbiprofen showed enantioselectivity for some pharmocokinetic parameters. Cl_B was higher for the (*S*)-(+)-flurbiprofen than for its antipode, as in calves. In cows in gestation, the kinetic behaviour of flurbiprofen did not show enantioespecificity. This difference may be due to different hormonal status in the two categories of cows. Li and colleagues (1999) demonstrated that multiple hormones (triiodothyronine, growth hormone, sex hormones) take part in the regulation of UGT mRNA expression in the rat. Triiodothyronine downregulates the periportal expression of alpha class glutathione *S*-transferase in rat liver (Vanhaecke *et al*., 2001). Furthermore, cows in early lactation in this work had a higher level of palmitic acid owing to fat mobilization (270.7 vs 239.36 mg% ($p = 0.06$), unpublished data). This suggest a higher availability of long-chain acyl-CoAs. Krcmery and Zakim (1993) suggested that long-chain acyl-CoAs could regulate the functional state of UDP-glucuronosyltransferase by inhibiting it.

Enantiomeric differences in the pharmacokinetics of chiral drugs are highly significant for therapeutic efficacy, since biological activity commonly resides in a single enantiomer and high eudismic ratios are the rule rather than the exception (Williams and Lee, 1985).

In conclusion, considering the differences in pharmacokinetic parameters of flurbiprofen found between calves and cows, the effects of physiological age and status (lactation, gestation) should be considered in the therapeutic use of chiral drugs.

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REFERENCES

Abas, A. and Meffin, P.J., 1987. Enantioselective disposition of 2-arylpropionic acid non-steroidal anti-inflammatory drugs. IV. Ketoprofen disposition. *Journal of Pharmacology and Experimental Therapeutics*, **240**, 637–641

Baggot, J.D., 1978. Some aspects of clinical pharmacokinetics in veterinary medicine. *Journal of Pharmacology and Experimental Therapeutics*, **1**, 111–118

Baggot, J.D., 1994. Drug therapy in the neonatal foal. *Veterinary Clinics of North America, Equine Practice*, **10**, 87–107

Brodgen, R.N, Heel, R.C., Speight, T.M. and Avery G.S., 1979. Flurbiprofen: a review of its pharmacological properties and therapeutic use in rheumatic disease. *Drugs*, **18**, 112–116

Caldwell, J., Hutt, A.J. and Fournel-Gigleux, S., 1988. The metabolic chiral inversion and dispositional enantioselectivity of the 2-arylpropionic acids and their biological consequences. *Biochemical Pharmacology*, **37**, 105–114

Conover, W.J., 1971. *Practical Nonparametric Statistics*, (Wiley, New York)

Crisman, M.V., Wilcke, J.R. and Sams, R.A., 1996. Pharmacokinetics of flunixin meglumine in healthy foals less than twenty-four hours old. *American Journal of Veterinary Research*, **57**, 1759–1761

Dannenberg, A.J. and Yang, E.K., 1992. Effect of dietary lipids on levels of UDP-glucuronosyltransferase in liver. *Biochemical Pharmacology*, **44**, 335–340

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

de Backer, P., 1986. Comparative neonatal pharmacokinetics. In: A.S.M.P.A.M. van Miert, M.G. Bogaert and M. DeBackere (eds), *Comparative Veterinary Pharmacology, Toxicology and Therapy*, (MTP Press, Lancaster), 161

Delatour, P., Foot, R., Foster, A.P., Baggot, D., Lees, P., 1996. Pharmacodynamics and chiral pharmacokinetics of carprofen in calves. *British Veterinary Journal*, **152**, 183–198

Eeckhoudt, S.L., Evrard, P.A. and Verbeeck, R.K., 1997. Biliary excretion and enterohepatic cycling of *R*- and *S*-flurbiprofen in the rat. *Drug Metabolism and Disposition*, **25**, 428–430

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

Geisslinger, G., Lótsch, J., Menzel S., Kobal, G. and Brune, K., 1994. Stereoselective disposition of flurbiprofen in healthy subjects following administration of the single enantiomers. *British Journal Clinical Pharmacology*, **37**, 392–394

Gibaldi , M. and Perrier, D., 1982. *Pharmacokinetics*, 2nd edn, (Marcel Dekker, New York)

Hamdoune, M., Mounie, J., Magdalou, J., Masmoudi, T., Goudonnet, H. and Escousse, A., 1995. Characterization of the *in vitro* glucuronidation of flurbiprofen enantiomers. *Drug Metabolism and Disposition*, **23**, 343–348

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

Jamali, F., Berry, B.W., Tehrani, M.R. and Russell, A.S., 1988. Stereoselective pharmacokinetics of flurbiprofen in human and rats. *Journal of Pharmaceutical Sciences*, **77**, 666–669

Kantor, T.G. 1986. Physiology and treatment of pain and inflammation. Analgesic effects of flurbiprofen. *American Journal of Medicine*, **80**, 3–9

Knadler, M.P. and Hall, S.D., 1989. High-performance liquid chromatographic analysis of the enantiomers of flurbiprofen and its metabolites in plasma and urine. *Journal of Chromatography, Biomedical Applications*, **494**, 173–182

Krcmery, M. and Zakim, D., 1993. Effects of oleoyl-CoA on the active and functional state of UDP-glucuronosyltransferase. *Biochemical Pharmacology*, **46**, 897–904

Li, Y.Q., Prentice, D.A., Howard, M.L., Mashford, M.L. and Desmond, P.V., 1999. The effect of hormone on the expression of five isoforms of UDP-glucuronosyltransferase in primary cultures of rat hepatocytes. *Pharmacology Research*, **16**, 191–197

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

Nouws, J.F.M., 1992. Pharmacokinetics in immature animals. *Journal of Animal Science*, **70**, 3627–3634

Reiche, R., 1983. Drug disposition in the newborn. In: Y. Ruckebush, P.L. Toutain and G.D. Koritz (eds), *Veterinary Pharmacology and Toxicology*, (MTP Press, Lancaster), 49

Risdall, P.C., Adams, S.S., Crampton, E.L. and Marchant, B., 1978. The disposition and metabolism of flurbiprofen in several species including man. *Xenobiotica*, **8**, 691–703

Sato, Y., 1997. Pharmacokinetics of antibiotics in neonates. *Acta Paediatrica Japonica*, **39**, 124–131

Shoaf, S.E., Schwark, W.S., Guard, Ch.L. and Babish, J.G., 1987. The development of hepatic drug-metabolizing enzyme activity in the neonatal calf and its effect on drug disposition. *Drug Metabolism and Disposition*, **15**, 676–681

Soraci, A., Benoit, E. and Delatour, P., 1995. Comparative metabolism of (R)-fenoprofen in rats and sheep. *Journal of Veterinary Pharmacology and Therapeutics*, **18**, 1–5

Szpunar, G.J., Albert, K.S, Bole, G.G., Dreyfus, J.N., Lockwood, G.F. and Wagner, J.G., 1987. Pharmacokinetics of flurbiprofen in man. I. Area/dose relationships. *Biopharmaceutics and Drug Disposition*, **8**, 273–283

Tracy, T.S., Rosenbluth, B.W., Wrighton, S.A., Gonzalez, F.J. and Korszekwa, K.R., 1995. Role of cytochrome P450 2C9 and an allelic variant in the 4′-hydroxylation of (*R*)- and (*S*)-flurbiprofen. *Biochemical Pharmacology*, **49**, 1269–1275

Tracy, T.S., Marra, C., Wrighton, S.A., Gonzalez, F.J. and Korszekwa, K.R., 1996. Studies of flurbiprofen 4′-hydroxylation. Additional evidence suggesting the sole involvement of cytochrome P450 2C9. *Biochemical Pharmacology*, **52**, 1305–1309

Vanhaecke, T., Lindros, K.O., Oinonen, T. Vercruysse, A. and Rogiers, V., 2001. Triiodothyronine downregulates the periportal expression of alpha class glutathione *S*-transferase in rat liver. *FEBS Letters*, **487**, 356–360

Wilcke, J.R., Crisman, M.V., Sams, R.A. and Gerken, D.F., 1993. Pharmacokinetics of phenylbutazone in neonatal foals. *American Journal of Veterinary Research*, **54**, 2064–2067

Wilcke, J.R., Crisman, M.V., Scarratt, W.K. and Sams, R.A., 1998. Pharmacokinetics of ketoprofen in healthy foals less than twenty-four hours old. *American Journal of Veterinary Research*, **59**, 90–292

Williams, K. and Lee, E.C., 1985. Importance of drug enantiomers in clinical pharmacology. *Drugs*, **30**, 333–354

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