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Some Pharmacokinetic Parameters of *R*-(–)- and *S*-(+)-Ketoprofen: The Influence of Age and Differing Physiological Status in Dairy Cattle

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ABSTRACT

The pharmacokinetic parameters of ketoprofen have previously been studied in cattle, but no studies have been performed on differing ages and metabolic situations in these animals. The aim of this work was to study the possible modifictions of the pharmacokinetics of ketoprofen enantiomers that may result from age, lactation or gestation in dairy cattle. Three groups of Holando Argentino cattle contained, respectively, 8 cows in early lactation, 8 pregnant cows and 8 newborn calves. Four animals from each group received the enantiomer *R*-(–)-ketoprofen, the other four animals received the *S*-(+) enantiomer, all by intravenous injection at a dose of 0.5 mg/kg. Significant differences between the three categories of animals were obtained in elimination half-life ($t_{\frac{1}{2}}$) (1.52, 0.87 and 0.31 and 1.71, 0.69 and 0.26 in newborn calves, cows in early lactation and cows in gestation, respectively), mean residence time (MRT) (0.45, 1.25, 2.20 and 0.38, 0.99, 2.47 h, in cows in gestation, cows in early lactation and newborn calves, respectively) and area under the plasma concentration-time curve (AUC) (0.87, 2.93, 3.24, and 0.67, 2.78, 5.13 (μg/h)/ml in cows in gestation, cows in early lactation and newborn calves, respectively, for the *R*-(–) and *S*-(+) enantiomer, respectively. In calves, there was a significant difference in AUC (3.24 vs 5.13 (μg h)/ml between *R*-(–)- and *S*-(+)-ketoprofen. In view of the differences between calves and adult cattle in the pharmacokinetic results for ketoprofen, the effects of age and physiological status (lactation, gestation) should be taken into account for therapeutic regimens.

Keywords: age, cattle, enantiomers, gestation, ketoprofen, lactation, pharmacokinetics, pre-ruminant

Abbreviations: *R*-(–) and *S*-(+), enantiomers of ketoprofen; AUC, area under curve; $t_{\frac{1}{2}}$, elimintion half-life; ClB, total body clearance; HPLC, high-performance liquid chromatography; MRT, mean residence time; V_{ss}, volume of distribution steady state

INTRODUCTION

Ketoprofen is a nonsteroidal anti-inflammatory drug (NSAID) of the 2-arylpropionic acid class. It contains an asymmetric carbon atom, so it exists in two enantiomeric forms, *S*-(+)- and *R*-(–)-ketoprofen. Several groups have investigated the pharmacokinetics of racemic ketoprofen in species of particular veterinary interest, including horses, dogs, cats, sheep, calves and camels (Delatour *et al.*, 1993; Jaussaud *et al.*, 1993; Benoit *et al.*, 1994; Landoni and Lees, 1995, 1996; Alkatheeri *et al.*, 1999). Chiral

inversion of ketoprofen has been demonstrated in various species, including rabbits, rats, cattle, dogs, cats, horses and humans (Abas and Meffin, 1987; Foster and Jamali, 1988; Foster *et al.*, 1988; Jamali *et al.*, 1990; Delatour *et al.*, 1993; Landoni and Lees, 1996; Igarza *et al.*, 2002). The pharmacokinetics of ketoprofen in cattle has been studied by Landoni and colleagues (1995) and by De Graves and colleagues (1996), while Igarza and colleagues (2002), in their study on chiral inversion of (*R*)-ketoprofen, described the plasma concentration–time profiles for both the *R*- and the *S*-enantiomers in pre-ruminant calves, cows in early lactation and cows in gestation.

The objective of this work was to study the possible modifications of the pharmacokinetics of ketoprofen enantiomers that may result from age, lactation or gestation in dairy cattle, following the administration of one or the other of the two enantiomers.

MATERIALS AND METHODS

Animals, drug administration and sampling

Twenty-four clinically normal, Holando Argentino cattle were used. They formed three distinct groups: 8 cows in early lactation (4–5 days postpartum), 8 cows in the sixth month of gestation, and 8 pre-ruminant calves (weighing 30 ± 3 kg) of 4–5 days of age. Four animals in each group received the *R*-(–) enantiomer (synthesized in the Chemistry Section of Laboratorie Menarini, Badalona, Spain; optical purity $>99\%$) by intravenous administration at a dose of 0.5 mg/kg. The other four calves in each group received the *S*-(+) enantiomer (synthesized in the Chemistry Section of Laboratories Menarini; optical purity $>99\%$) at the same dose. Blood samples were collected from each animal in all three groups at 5, 10, 15 and 30 min, and then at intervals of 30 min until 1.5, 2 and 4 h after dosing. 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 ethyl acetate, which was subsequently evaporated to dryness under a 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.*, 1995). This procedure converts the enantiomers into diastereoisomers, which can be analysed on classic HPLC reversed-phase columns. To accomplish the conversion, 100 µl triethylamine 50 mmol/L (in acetonitrile), 50 µl ethyl chloroformate 60 mmol/L (in acetonitrile), 50 µl L-leucinamide hydrochloride 1 mol/L (in water), 50 µl triethylamine 1 mol/L (in methanol) and 50 µl water were successively added to the dry extract. The diasteroisomers so produced were resolved by HPLC. The HPLC gradient system consisted of a LKB (Pharmacia, Bromma, Sweden) pump, model 2249, UV variable detector model 2141 and software HPLC manager. The compounds were eluted from a RP 18 column

(0.4 × 15 cm, 5 μm particles size) with a mobile phase of acetonitrile–K_2PO_4 10 mmol/L mixture and a gradient of from 35–50% over 16 min at a flow rate of 1.5 ml/min. Detection was by UV at 252 mm. Under these conditions, the retention times for *R*-(–)- and *S*-(+)-ketoprofen were 6.4 and 6.9 min, respectively. Detection was linear for the two antipodes between 0.25 μg/ml and 10 μg/ml. The percentage of *R*-ketoprofen recovered was 87% and that of *S*-ketoprofen was 86%. The limit of quantification was 0.2 μg/ml. The coefficient of variation for the assay was 5%.

Data analysis

Pharmacokinetic analysis of the plasma disposition curves of *S*-(+)- and *R*-(–)-ketoprofen in each animal was carried out by non-linear least-square regression analysis using the computer program PCNONLIN (Statistical Consultants, Lexington, KY, USA). The best-fitting model was discriminated by applying the Akaike criterion using the MAICE (minimum Akaike information criterion estimation) test (Akaike, 1976; Yamaoka *et al.*, 1978). The area under the curve (AUC) for both *R*-(–)- and *S*-(+)-ketoprofen was measured 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. The apparent volume of distribution at steady state and the body clearance were calculated by classical methods (Baggot, 1978; Gibaldi and Perrier, 1982).

Statistical analysis

Data are expressed as median, minimum and maximum. Statistical comparisons between the different categories of animals and the different enantiomers of ketoprofen for the pharmacokinetic parameters were carried out by applying the Kruskal–Wallis test and Wilcoxon two-sample test (Conover, 1971), using the NPAR1WAY procedure of Statistical Analysis Systems, Version 6 (SAS, Institute Inc., Cary, NC, USA). Differences were considered to be statistically significant if $p < 0.05$.

RESULTS

The pharmacokinetic parameters for cows in gestation, cows in early lactation and calves are shown in Table I. The volume of distribution at steady state (V_{ss}) for both enantiomers was low, with no differences between the different groups of animals. A statistically significant difference between each of the three categories of animals was found for both enantiomers in elimination half-life ($t_{\frac{1}{2}}$), which was longer for newborn calves than for cows in early lactation and shortest for cows in gestation. Total body clearance (ClB) was more rapid for cows in gestation than for cows in early lactation or calves for both enantiomers, but the difference between the three groups was only significant for the *S*-(+) enantiomer. This agrees with the longer mean residence

TABLE I
Stereoselective pharmacokinetic parameters (median, minimum–maximum) for *R*-(–)- and *S*-(–)-ketoprofen obtained after intravenous administration of 0.5 mg/kg of *R*-(–)- or *S*-(–)-ketoprofen to pre-ruminant calves, cows in early lactation and cows in gestation

Pharmacokinetic parameter		Cows in gestation ($n = 4$)	Cows in early lactation ($n = 4$)	Newborn calves ($n = 4$)
AUC ((μg h)/ml)	*R*	0.78 (0.56–1.35)[a]	3.00 (1.49–4.21)[b]	3.39 (2.60–3.58)[b]*
	S	0.64 (0.52–0.89)[a]	2.75 (1.55–4.06)[b]	5.05 (3.76–6.66)[c]
$t_{\frac{1}{2}}$ (h)	*R*	0.33 (0.23–0.35)[a]	0.91 (0.69–0.96)[b]	1.35 (1.09–2.30)[c]
	S	0.26 (0.20–0.33)[a]	0.71 (0.51–0.82)[b]	1.70 (1.28–2.15)[c]
ClB ((ml/h)/kg)	*R*	0.64 (0.37–0.90)[a]	0.18 (0.12–0.34)[b]	0.15 (0.1.4–0.19)[b]*
	S	0.78 (0.56–0.96)[a]	0.19 (0.12–0.32)[b]	0.10 (0.08–0.13)[c]
MRT (h)	*R*	0.48 (0.33–0.51)[a]	1.32 (1.00–1.38)[b]	1.95 (1.57–3.32)[c]
	S	0.37 (0.29–0.47)[a]	1.02 (0.74–1.19)[b]	2.46 (1.85–3.10)[c]
V_{ss} (L/kg)	*R*	0.33 (0.12–0.40)[a]	0.25 (0.15–0.34)[a]	0.30 (0.28–0.46)[a]
	S	0.28 (0.27–0.31)[a]	0.19 (0.15–0.24)[a]	0.25 (0.19–0.31)[a]

AUC, area under curve; $t_{\frac{1}{2}}$, elimination half-life; ClB, total body clearance; MRT, mean residence time; V_{ss}, volume of distribution at steady state

R, *R*-(–)-ketoprofen; *S*, *S*-(–)-ketoprofen

[a,b,c]Different letters indicate significant differences between groups of animals (rows): $p < 0.05$

*Significant differenc between the *R*-(–) and *S*-(+) enantiomers: $p < 0.05$

(MRT) for newborn calves than for cows in early lactation and for cows in gestation (in reducing order).

The values of the area under the plasma concentration–time curve (AUC) were lower for cows in gestation than for cows in early lactation and calves but this was only significant for the *S*-(+) enantiomer.

There were no significant differences between *R*-(–) and *S*-(–)-ketoprofen in any pharmacokinetic parameter in cows, indicating that enantioselective pharmacokinetics did not occur in adult animals after intravenous administration of the *R*-(–) or *S*-(+) enantiomer. However, in newborn calves, there was a significant difference between *R*-(–)- and *S*-(–)-ketoprofen, in that the AUC was longer and ClB was lower for the (*S*) enantiomer, indicating that there was enantioselective behaviour in pre-ruminant calves (Table I).

DISCUSSION

Differing enantioselective pharmacokinetic behaviour of ketoprofen under different physiological situations in one species has been clearly demonstrated in this study. A previous report described differences in the chiral inversion of *R*-(–)-ketoprofen in cattle at different ages and in different physiological situations (Igarza *et al.*, 2002). Chiral inversion was higher in newborn calves than in cows in early lactation or cows in gestation (50.5%, 33.3% and 26%, respectively). The chiral inversion previously observed in ruminating calves was 31% (Landoni and Lees, 1995).

The metabolism of ketoprofen consists of three biotransformations: (a) conjugation to an acylglucuronide, (b) hydroxylation on the aromatic ring of the benzoic group, and (c) inversion of the *R*-(–) to the *S*-(+) enantiomer (Mauleón *et al.*, 1996). While the first process of glucuronidation is the major transformation in all the species studied, the relevance of the other two biotransformations varies considerably between species, according to their age and different physiological situations (Satterwhite and Boudinot, 1992; Aberg *et al.*, 1995; Igarza *et al.*, 2002). As the chiral inversion in cattle was higher in calves than in cows, this could contribute to the difference in the pharmacokinetic parameters between newborn and adult animals. Newborn calves have markedly less drug-metabolizing enzymes than adults, so clearance of drugs that require hepatic metabolism may be delayed (Kawalek and El Said, 1994). For instance, the activity of UDP-glucuronyltransferase reaches adult values by 7 days of age, but the overall ability to glucuronidate chloramphenicol increases after this age because the microsomal protein content continues to increase (Shoaf *et al.*, 1987).

Some pharmacokinetic differences in dairy cows were also demonstrated between those in early lactation and those in gestation. The lower clearance in early lactation could be due to the influence of the growth hormone, which suppresses expression of glucuronyltransferase. Thus, incubation of growth hormone with rat hepatocytes suppressed the expression of glucuronyltransferase (Guéraud and Paris, 1998; Li *et al.*, 1999). The $t_{\frac{1}{2}}$ for ketoprofen observed in this study was shorter in adult cattle than in some other species as it is approximately 2 h for humans (Williams and Upton, 1988) and approximately 4 h for dogs (Schmitt and Guentert, 1990), but it was similar to that in horses of approximately 25 min (Jaussaud *et al.*, 1993). Moreover, the value in newborn calves (non-ruminant) was nearer to that for monogastric animals.

Enantiomeric differences in the pharmacokinetics of chiral drugs are highly relevant to their 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). Enantioselectivity occurred in calves, AUC being longer for the *S*-(+) enantiomer than for the *R*-(–) enantiomer, while ClB was lower for the *S*-(+) enantiomer than for the *R*-(–) enantiomer. The enantioselectivity is due to the unidirectional chiral inversion of *R*-(–)- to *S*-(+)-ketoprofen in calves (50.5%) (Igarza *et al.*, 2002). In other species, including the rat, in which the (*S*)/(*R*) ratio is 11:8.1 (Foster and Jamali, 1988), and horses, in which the (*S*)/(*R*) ratio is 1.5:1 (Jaussaud *et al.*, 1993; Landoni and Lees, 1996), enantioselective pharmacokinetics have been described. However, although enantioselectivity occurred in calves, the pharmacokinetic parameters in cows were similar for both enantiomers, indicating a lack of

enantioselective pharmacokinetics. Similar results were obtained with ruminant calves (Landoni *et al.*, 1995). The metabolic processing of the *R*-(–) enantiomer (hydroxylation, chiral inversion and conjugation) and of the *S*-(+) enantiomer (hydroxylation and glucuronoconjugation) may be balanced due to a lower chiral inversion of (*R*) to (*S*) in cows than in calves. This could explain the absence of enantioselectivity in the various pharmacokinetic parameters in cows.

Hence, the effects of physiological age and status (lactation, gestation) should be taken into account in designing therapeutic regimens for use in cattle.

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