Chiral inversion of R(−) fenoprofen and ketoprofen enantiomers in cats

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The chiral inversion process is a characteristic metabolic pathway for different aryl-2-propionic acids or profens. Important variations have been observed between these individual compounds as well as between animal species. In this study, R(−) fenoprofen [R(−)FPF] and R(−) ketoprofen [R(−) KTF] were used to investigate their comparative stereoconversion in cats. After intravenous (i.v.) administration of R(−) FPF, the percentage of chiral inversion was 93.20 ± 13.70%. A highly significant correlation (r: 0.978) was observed between the clearance of R(−) FPF and the chiral inversion process. After i.v. administration of R(−) KTF, the percentage of inversion was only 36.73 ± 2.8%. No correlation between the clearance of R(−) KTF and this process was observed. R(−) FPF was metabolized by the pathways of thioesterification – chiral inversion processes. For R(−) KTF, the competitive metabolic pathways, glucuronidation and hydroxylation may be involved. However, these metabolic steps are saturable or less functional in cats. Moreover, the thioesterification of R(−) KTF in *in vitro* studies has been shown to be important in carnivores. The lack of correlation between clearance and chiral inversion process of R(−) KTF may be finally explained by deviation of thioesterification to other metabolic pathways of lipids and/or aminoacid conjugation, particulary glicine derivatives.

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INTRODUCTION

Fenoprofen (FPF) and ketoprofen (KTF) (Fig. 1) are two non-steroidal anti-inflammatory compounds of the 2-arylpropionic acid class. These chiral compounds are marketed as racemates and used for the treatment of osteoarthritis, postoperative analgesia and as antipyretic (Cabre *et al.*, 1998; Slingsby & Waterman-Pearson, 1998). Both drugs contain a chiral carbon (C2) and therefore exist as two non-superimposable mirror-image forms or R(−) and S(+) enantiomers.

In vitro studies on the relative anti-inflammatory activity of individual FPF and KTF enantiomers have shown that their effect on cyclo-oxygenase is due to the S(+) enantiomer (Hutt & Caldwell, 1983; Evans, 1992). The two asymmetric compounds often have different pharmacological potencies associated with stereoselective behaviours (Evans, 1992). Among them, the metabolic chiral inversion process has considerable therapeutic significance. This process of biotransformation corresponds to a selective unidirectional transformation from the inactive R(−) to the active S(+) enantiomer. The stereoconversion mechanism has been described in different organs such as liver, intestine, kidney and lungs (Cox *et al.*, 1985; Jeffrey *et al.*, 1991; Hall *et al.*, 1992). However, the liver seems to have the predominant role in the chiral inversion process (Berry & Jamali, 1991).

The molecular development of the chiral inversion mechanism has been described by several authors (Wechter *et al.*, 1974; Nakamura *et al.*, 1981; Knihinicki *et al.*, 1989; Menzel *et al.*, 1994). Three steps are involved in this process: (i) activation of the R(−) profen by the formation of acyl-coenzyme A thioester; (ii) enzymatic epimerization of the R(−) thioester to the S(+) thioester/or hydrolysis to regenerate the R(−) enantiomer; and in the final step (iii), hydrolysis of the S thioester completes the inversion process (Fig. 2).

The chiral inversion of FPF and KTF has been documented in a variety of species. However, there are no stereoselective metabolic studies available for these compounds in cats. The aim of this study was to determine the comparative chiral inversion of FPF and KTF in cats.

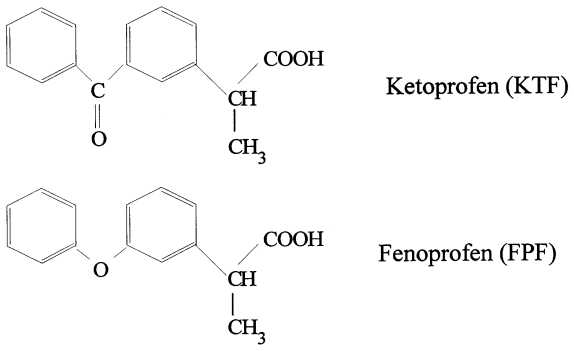

Fig. 1. Chemical structure of KTF and FPF.

MATERIALS AND METHODS

Chemicals

FPF racemic was obtained from Sigma (Fenoprofen calcium salt, hydrate; St Louis, MO, USA). The R(−) and S(+) enantiomers of FPF were obtained by stereospecific crystallization, using α-methylbenzylamine as a chiral inducer (Hayball & Meffin, 1987). After completing the crystallization process, the final purities determined by high-performance liquid chromatography (HPLC) for the R(−) and S(+) enantiomers were 98.6 and 98.0%, respectively. The R(−) and S(+) enantiomers of KTF were kindly supplied by Laboratorios Menarini S.A., Badalona, Spain. L-leucinamide and α-methylbenzylamine were purchased from Fluka, SA, Saint-Quentin, France. All other chemical reagents were obtained from the usual commercial sources.

Animals and experimental protocol

Two groups of four adult cats weighing from 3.8 to 4.1 kg were used. Following an acclimatization period of at least 3 weeks, the cats were anaesthetized and the right jugular vein was catheterized according to the technique previously described (Castro *et al.*, 1998). After recovery from the anaesthesia, one group was given R(−) FPF and the other one R(−) KTF at a dose of 1 mg/kg intravenously (i.v.). Seven days later, each group received S(+) FPF and S(+) KTF at the same doses, respectively. The enantiomers were dissolved in a mixture of 200 µL DMSO and 800 µL physiological solution. Blood samples were collected at 5, 10, 20 and 30 min and 1, 2, 4, 6 and 8 h after the administration of FPF and KTF enantiomers. Samples of 5 mL each were centrifuged and the plasma was separated and stored at −20°C until analysis.

Analytical method

FPF enantiomers were extracted from the plasma using Sep-Pack cartridges C18 in accordance with a method described by Castro *et al.*, 1998. For the KTF enantiomer extraction, 0.5-mL plasma aliquots were acidified with HCl (1 N) and extracted twice with 6 mL diethyloxide (Benoit *et al.*, 1994; Delatour *et*

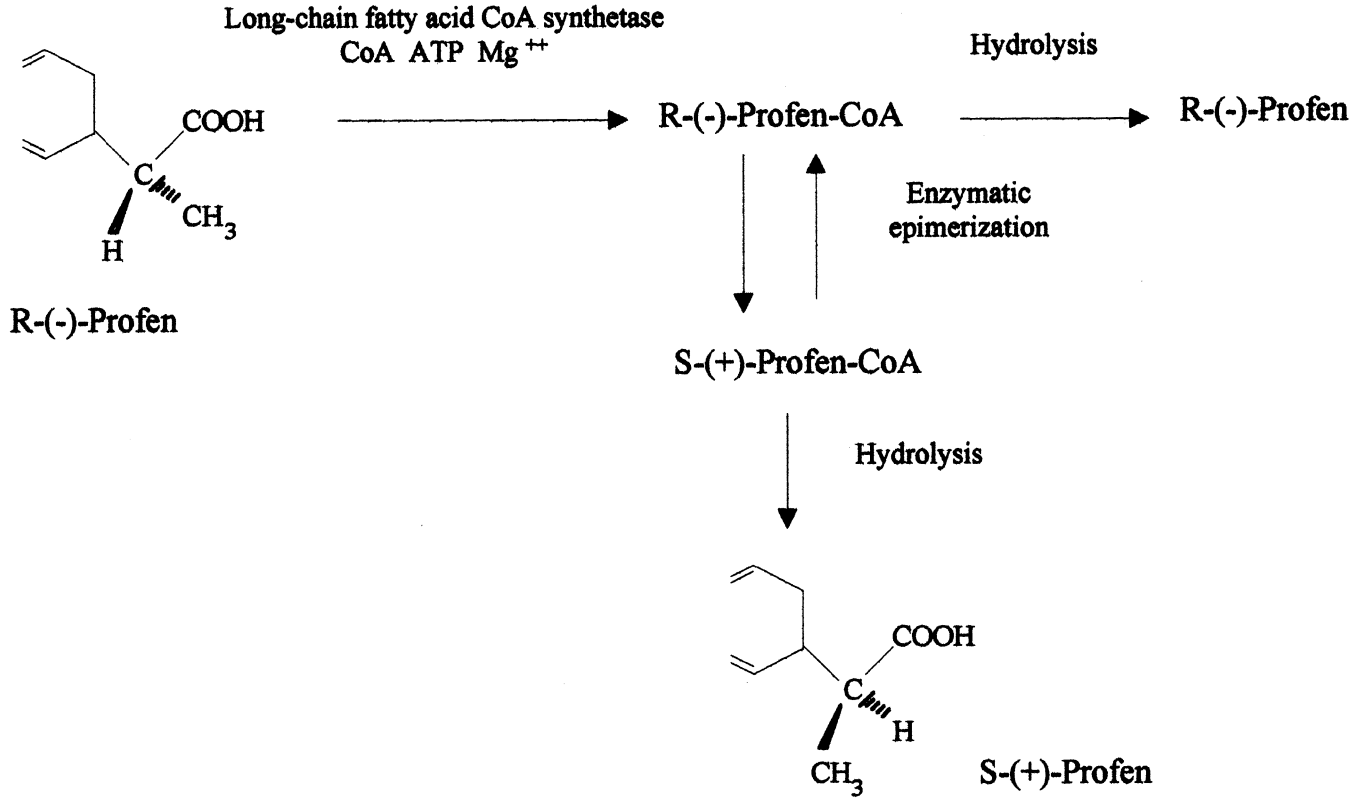

Fig. 2. Chiral inversion process of aryl-2-propionic acids or profens.

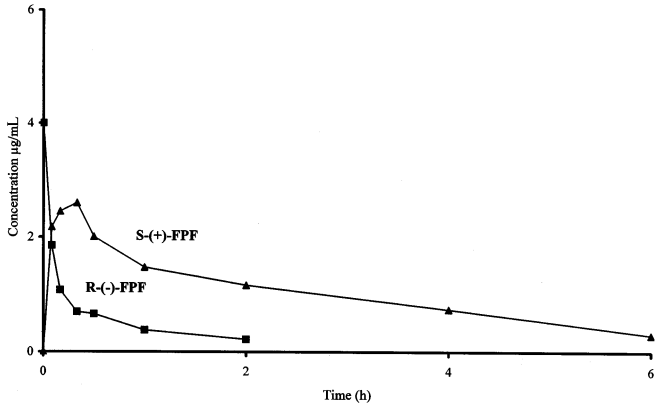

Fig. 3. Mean plasma concentration–time curve of FPF enantiomers in cats after i.v. administration of 1 mg/kg of R(−) FPF.

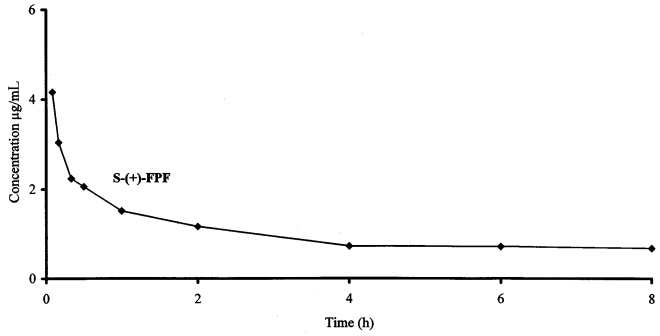

Fig. 4. Mean plasma concentration–time curve of S(+) FPF enantiomer in cats after i.v. administration of 1 mg/kg of S(+) FPF.

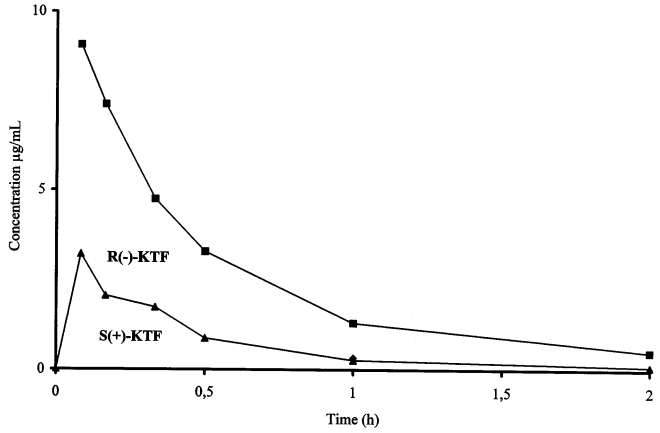

Fig. 5. Mean plasma concentration–time curve of KTF enantiomers in cats after i.v. administration of 1 mg/kg of R(−) KTF.

al., 1994a,b; Soraci *et al.*, 1995; Soraci, 1995). The organic extract obtained from the two extractions was evaporated to dryness under a nitrogen stream. The dry residue was derivatized with L-leucinamide (Foster & Jamali, 1987). The diastereomers thus produced were resolved by HPLC according to the method described by Soraci *et al.* (1995).

Data analysis

Pharmacokinetic parameters were estimated using non-compartmental methods and were fitted using a computer program (PK Solution®; Ashland, OH, USA) for each animal after i.v. administration of FPF and KTF enantiomers. The areas under the curves (*AUC*) were determined by the trapezoidal rule (Baggot, 1978). The enantiomeric conversion of R(−) FPF and R(−) KTF into their respective S(+) antipodes was calculated using the formula of Pang & Kwan (1993):

Inversion rate: $AUC_{(S)\text{ after }(R)} \times \text{dose (S)}/AUC_{(S)\text{ after }(S)} \times \text{Dose (R)}$

Kinetic parameter comparisons of FPF and KTF enantiomers were performed using an unpaired *t*-test (StartGraph®). Significance was accepted at $P < 0.05$.

RESULTS

Mean plasma concentrations of FPF enantiomers after R(−) FPF and S(+) FPF administration are shown in Figs 3 and 4, respectively. No trace of R(−) FPF enantiomers could be detected after S(+) FPF administration. On the contrary, after R(−) FPF dosage, the plasma concentration of S(+) enantiomer exceeded that of R(−) as early as 15 min after dosage. The calculated stereoconversion rate was $93.20 \pm 13.7\%$. The S(+) FPF concentrations then slowly decreased. The inversion rate of R(−) into S(+) KTF after i.v. administration of R(−) enantiomer was only $36.73 \pm 2.8\%$ (Fig. 5). No chiral inversion from S(+) to R(−) enantiomer was detected after the administration of S(+) KTF (Fig. 6). Several pharmacokinetic parameter values of FPF and KTF enantiomers are present in Table 1. There were significant differences between the R(−) KTF and R(+) FPF enantiomers for the clearance and *AUC* parameters. No statistically significant difference between both R(−) enantiomers was obtained in elimination half-life. However, after the administration of S(+) enantiomer of these compounds, the mean elimination half-life of S(+) FPF was longer than that of S(+) KPF. A statistical significance ($P < 0.05$) was found between all kinetic parameters [$AUC_{(0-T)}$, CL_b, $T_{1/2\ el}$, $AUC_{(0-T)}$ S(+) after R(−) and $T_{1/2\ el}$ S(+) after R(−) for FPF and KTF enantiomers] of S(+) KTF and S(+) FPF considered (Table 1).

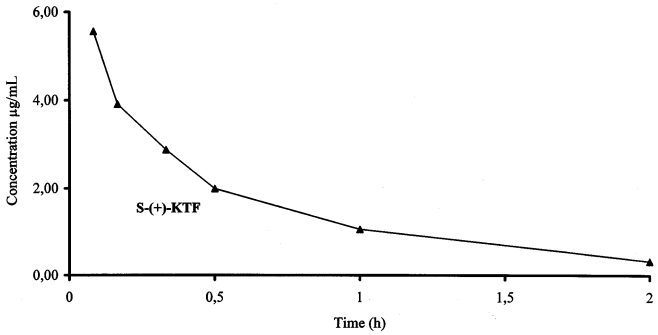

Fig. 6. Mean plasma concentration–time curve of S(+) KTF enantiomer in cats after i.v. administration of 1 mg/kg of S(+) KTF.

Table 1. Some mean pharmacokinetic parameters of FPF and KPF enantiomers obtained after i.v. administration of each enantiomer at a dose level of 1 mg/kg in cats

Parameters	R(−) FPF	R(−) KTF	S(+) FPF	S(+) KTF
$AUC_{(0-T)}$ (μg/h per mL)	1.125 ± 0.55*	4.03 ± 1.42	6.45 ± 1.90‡	3.85 ± 1.40
$CL_{(B)}$ mL/h per kg	978.6 ± 197.8†	235 ± 77.0	117.7 ± 39.5§	216.9 ± 60.6
$T_{1/2\ el}$ h	0.53 ± 0.11	0.50 ± 0.17	2.85 ± 1.81¶	0.52 ± 0.11
$AUC_{(0-T)}$ (μg/h per mL) $S(+)_{after}$ R(−)			5.62 ± 0.80**	1.47 ± 0.40
$T_{1/2\ el}$ h $S(+)_{after}$ R(−)			3.20 ± 0.60††	0.35 ± 0.10

Significantly different ($P<0.05$). *AUC R(−) FPF and R(−) KTF: P value of 0.0322. †$CL_{(B)}$ R(−) FPF and R(−) KTF: P value of 0.0209. ‡AUC S(+) FPF and S(+) KTF: P value of 0.0267. §$CL_{(B)}$ S(+) FPF and S(+) KTF: P value of 0.0339. ¶$T_{1/2\ el}$ S(+) FPF and S(+) KTF: P value of 0.0153. **AUC $S(+)_{after}$ R(−) P value of 0.0001. ††$T_{1/2\ el}$ h $S(+)_{after}$ R(−) P value of 0.0001.

DISCUSSION

The enantiomeric disposition kinetic data for FPF have been described in humans (Rubin *et al.*, 1985), rats (Berry & Jamali, 1991), rabbits (Hayball & Meffin, 1987), sheep (Soraci *et al.*, 1995), dogs and horses (Soraci *et al.*, 1996). The enantioselective disposition data for KTF have been described in horses (Jaussaud *et al.*, 1993; Landoni & Lees, 1995a, 1996), dogs (Delatour *et al.*, 1994a; Soraci, 1995), calves (Landoni & Lees, 1995b), humans (Foster *et al.*, 1988; Hayball *et al.*, 1993) and several laboratory species. Stereospecific variations in pharmacokinetic data of FPF and KTP were observed in all the abovementioned species. Recently, we have observed that the mean S/R ratio for *AUC* of FPF racemic in cats after i.v. administration was 8.26. (Castro *et al.*, 1998).

These specific differences were generally associated with metabolic inversion of the chiral center (Hutt & Caldwell, 1983). In this way, our experimental study clearly confirms that the stereoselective pharmacokinetics of FPF observed was due to the unidirectional chiral inversion of R(−) FPF to the S(+) antipode. An important difference in the stereoconversion rate for these closely related compounds (FPF and KTF) was observed in cats. The i.v. administration of R(−) FPF to cats showed a large chiral inversion (92.3%). A highly significant correlation (r: 0.978) between the clearance of R(−) FPF and the chiral inversion process strongly suggests the involvement of stereoconversion as the main metabolic pathway for R(−) FPF in cats. A similar result has been reported for dogs (Soraci *et al.*, 1996).

This stereoselective relationship for FPF among these domestic carnivores could be explained at the molecular level. The activity of long chain fatty acid coenzyme A synthetase (EC 6.2.1.3), particularly the enzyme palmitoyl coenzyme A (CoA) ligase, involves the limiting step of the chiral inversion process (Knigths & Jones, 1992; Tracy *et al.*, 1993). The expression of this enzyme is regulated by the level of lipids in the diet (Suzuki *et al.*, 1990). This enzyme presents a high capacity for its physiologic substrate (palmitic acid) and also for different aryl-

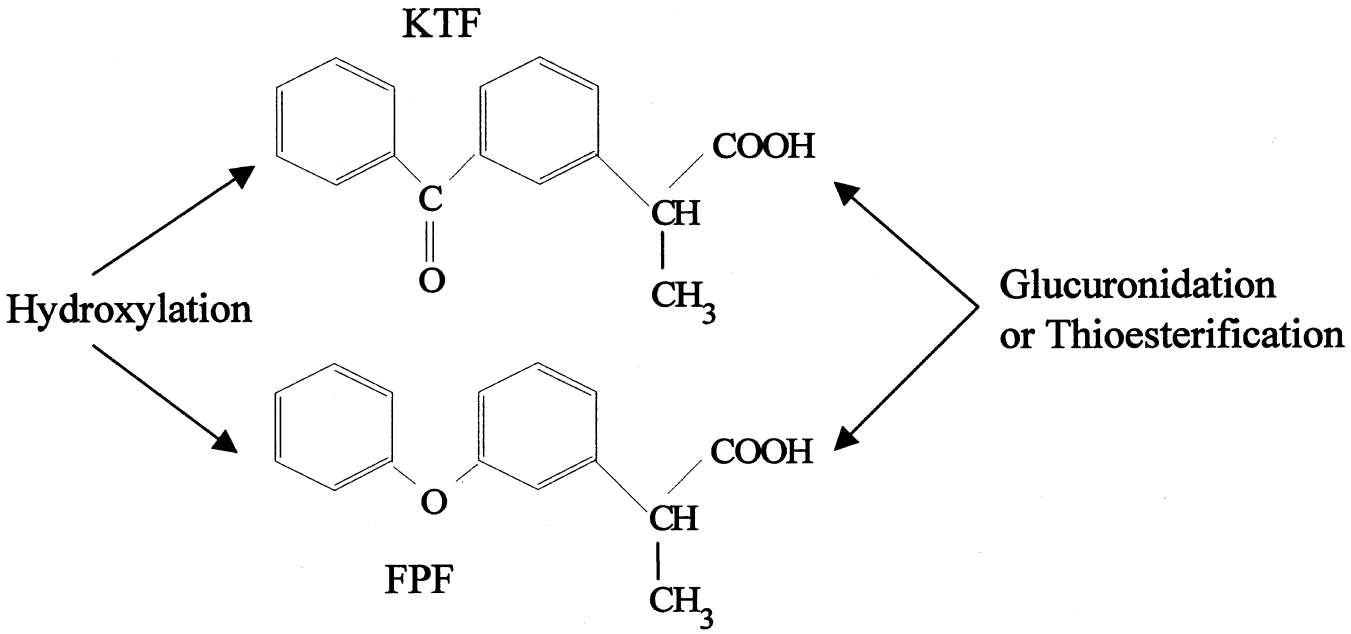

Fig. 7. Metabolic pathway of aryl-2-propionic acids.

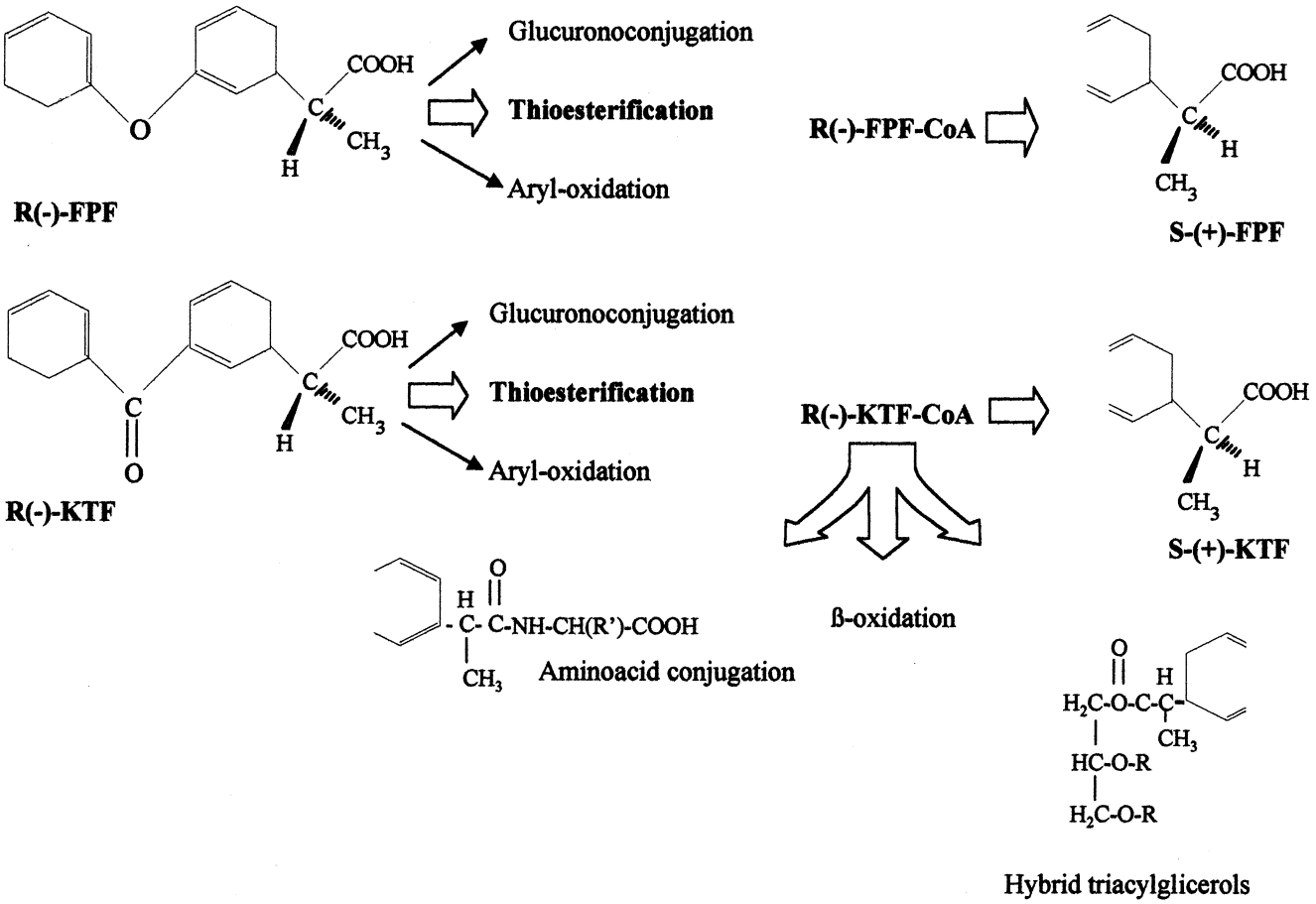

Fig. 8. Predominant metabolic pathways of R(−) FPF and R(−) KTF in cats.

carboxyl-xenobiotics in dogs (Benoit *et al.*, 1994; Soraci & Benoit, 1995). Concerning this, *in vitro* studies on dog liver microsomes showed high V_{max} values (60.6 ± 11 nmol/mg per min) for the thiosterification of R(−) FPF (Soraci & Benoit, 1995). Thus, the results reported here suggest the important role of R(−) FPF substrate for acyl-CoA synthetase, as well as its consecutive consumption through the pathway of chiral inversion in cats. However, to reach a definite conclusion regarding the role of R(−) FPF substrate for acyl-CoA synthetase, further *in vitro* thiosterification studies would be necessary.

The i.v. administration of R(−) KTF to cats showed a moderate chiral inversion (36.73%). This value was similar to those reported in dogs (Delatour *et al.*, 1994a; Delatour *et al.*, 1994b) and other species (Landoni & Lees, 1995a,b). The statistical difference observed between S(+) FPF and S(+)KTF after R(−) administration of FPF and KTF, respectively, could determine a greater therapeutic and/or potential toxic activity of racemic FPF than KTF in cats.

A low inversion rate for R(−) KTF compared to that of R(−) FPF and a lack of correlation between the clearance of R(−) KTF and this process were observed in cats. These findings could be explained by the utilization of the R(−) KTF enantiomer as a substrate by other competitive pathways, such as aryl oxydation, glucuronic conjugation or thioesterification (Fig. 7) (Jamali, 1988; Soraci *et al.*, 1995). Considering that the glucuronic conjugation is an easily saturable or less functional pathway in cats (Boutin *et al.*, 1981; Court & Greenblatt, 1997a,b) and that the capacity to hydroxylate acyl xenobiotics is low (Maugras & Reichart, 1979), the thioesterification could be the principal metabolic step for R(−) KTF in cats (Fig. 8), and oxidative processes and glucuronidation may only contribute to the modulation of the plasma profiles. Comparative *in vitro* studies carried out with microsome preparations from different animal species showed that carnivorous microsome produced the highest thiosterification of R(−) KTF (Delatour *et al.*, 1994a; Soraci, 1995; Sevoz *et al.*, 1997). Therefore, the lack of correlation between clearance and the chiral inversion process could be explained by the diversion of the intermediate thioester (R(−) KTF-CoA) towards other metabolic pathways, such as the metabolism of lipids with the formation of hybrid triacylglicerides, alteration of the β-oxidation (Fears *et al.*, 1978; Caldwell & Marsh, 1983; Williams *et al.*, 1986; Sallustio *et al.*, 1987; Moorhouse *et al.*, 1991; Zhao *et al.*, 1992; Hall & Quan, 1994; Knigths, 1998) and/or amino acid conjugation, particularly, glicine derivatives (Caldwell, 1978, 1982; Tanaka *et al.*, 1992; Knigths, 1998) (Fig. 8). Moreover, studies carried out with phenoxy-3-benzoic acid in cats showed that the conjugate

with glicine constitutes the main metabolite (Huckle *et al.*, 1981). However, further investigations should be made in order to demonstrate this hypothesis.

This study demonstrates that there is a high enantioselective pressure within the same species, even for chemically similar compounds, given the important specificity of substrate of the enzymes participating in the different metabolic processes involved. This imposes, in the veterinary practice, a great therapeutic and toxicological caution at the moment of extrapolating dose between different compounds.

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