The Veterinary Journal 2001, **162,** 38–43
doi: 10.1054/tvjl.2001.0584, available online at http://www.idealibrary.com on IDEAL®

Disposition of Suprofen Enantiomers in the Cat

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SUMMARY

Suprofen (SPF) is a non-steroidal anti-inflammatory drug (NSAID), which belongs to the 2-arylpropionic acids subclass. As a result of their chiral characteristics, these compounds have shown a marked enantioselective behaviour with a high degree of interspecies variation. They are mainly eliminated by glucuronidation. Plasma, biliary and urine disposition of SPF was investigated in the cat after intravenous administration of the racemate (dose 2 mg/kg). Both enantiomers exhibited similar disposition profiles in plasma with no evidence of chiral inversion. During bile sampling time, recovered acylglucuronides of R (–) and S (+) SPF were less than 1% of the total dose administered. Only free SPF was recovered in the urine, representing 0.12% of the administered racemic SPF dose. The results indicate that neither chiral inversion nor glucuronidation predominate in SPF disposition in cats.

KEY WORDS: Suprofen; cats; enantioselective; chiral inversion; glucuronidation.

INTRODUCTION

Suprofen (SPF) (α-methyl-p-[2-thenoyl]-phenylacetic acid) is a non-steroidal anti-inflammatory drug (NSAID) which belongs to the main group of available NSAIDs with a high degree of chemical homogeneity, the 2-arylpropionic acid derivatives or 'profens' (Deschamps-Labat *et al.*, 1997). This group contains an asymmetric carbon atom, a chiral centre located at the C-2 of the propionic moiety and, therefore, exists in two enantiomeric forms, R (–) and S (+). Only the (S) enantiomer has significant pharmacological activity on cyclooxygenase (Caldwell *et al.*, 1988; Yasui *et al.*, 1996). The structural characteristics of SPF can influence its biological fate and the disposition of SPF enantiomers may be enantioselective, whereby metabolic chiral inversion transforms the inactive (R)-enantiomer into the pharmacologically active (S)-form (Nakamura *et al.*, 1981). The key molecular basis for this mechanism involves the enantioselective formation of the coenzyme A (CoA) thioester by long chain CoA ligase (Sevoz *et al.*, 2000). The extent of inversion is different for each profen and is species dependent and usually unidirectional (Soraci *et al.*, 1995; Jamali *et al.*, 1997). Because of this variability, specific studies must be carried out in each species to elucidate the pharmacological and toxicological events related to the use of these drugs.

Glucuronidation is another potentially enantioselective process. This reaction is catalysed by uridine - diphosphate - glucuronosyltransferases (UDPGT) and leads to the formation of two 1-*O*-acyl-β-D-glucuronide diasteroisomers. UDPGT has been shown to exhibit similar Michaelis constant (K_m), but different maximal rate (V_{max}) values for R (–) and S (+) enantiomers *in vitro*. This suggests that the ability of the enzyme to conjugate glucuronic acid with the substrate depends on the conformational presentation of each enantiomer to

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1090-0233/01/010038 + 06 $35.00/0

the active site of the enzyme (Volland & Benet, 1991). The enantioselectivity of this mechanism is reported to be species dependent (Maire-Gauthier *et al.*, 1998). Although glucuronidation is the major metabolic pathway for this class of drugs in most mammalian species (Hutt & Caldwell, 1983; Volland & Benet, 1991; Smith & Liuh, 1993; Soraci *et al.*, 1995, Terrier *et al.*, 1999), the cat could be an exception, because of its inability to form glucuronides of phenolic and carboxilic compounds. As a consequence, cats are highly susceptible to the toxic side effects of many drugs, including 2-arylpropionic acids (Baggot, 1977; Alvarez & Pratt, 1990). The aim of the present study was to investigate the plasma, bile and urine disposition of racemic (*rac*) SPF in the cat.

MATERIALS AND METHODS

Suprofen was obtained from Sigma. L-leucinamide was purchased from ICN Pharmaceuticals. All other chemicals and reagents were obtained from usual commercial sources.

High-performance liquid chromatography (HPLC) was performed using a gradient system (LKB, Pharmacia), pump model 2949, UV variable detector model 2141 and software HPLC manager. The flow rate was 1.5 mL/min. The UV detector was set at 292 nm. The diasteroisomer forms of SPF were eluted from an RP 18 column (0.4 × 15 cm, 5 μm particle size) with a binary gradient, A: phosphoric acid (1%), B: acetonitrile. The retention times for R (–) and S (+) SPF were 7.15 and 8.23 min respectively. No interfering peaks were observed from any of the endogenous components of plasma, bile and urine. The recovery percentages of R (–) and S (+) SPF were 100% (plasma), 83.5–96.5% (bile) and 80–79% (urine). A linear relationship between the peak area ratios and the corresponding concentration of each enantiomer was observed from 0.2 to 10 μg/mL. The limit of detection was 0.05 μg/mL. The limit of quantification of SPF for different biological matrix was 0.2 μg/mL and the coefficient of variation was 5%.

Four healthy male cats (4.1 ± 0.18 kg body weight) aged eight months to two years were used. Cats were anaesthetized by an intravenous (i.v.) injection of ketamine (ketamine 50 mg/mL, Holliday-Scott S.A.) at a dose of 7 mg/kg. A K33 teflon catheter (Rivero S.A.) was placed in the right jugular vein. The catheter was fixed to the skin and the dead space was filled with heparinized saline. All cats also had a K33 catheter placed in the bile duct. The catheter was anchored subcutaneously, externalized at the right flank and connected to a collection flask kept in dry ice and placed below the anatomic plane of the gallbladder. After the recovery from anaesthesia each cat received a single i.v. dose of 2 mg/kg of *rac*-SPF. The drug was dissolved in a mixture of 300 μL of dimethyl sulphoxide (DMSO) and 300 μL of physiological saline solution.

Blood samples (2 mL) were collected from a jugular vein just before *rac*-SPF administration and at 5, 10, 20, 30 min and 1, 2, 4 h after treatment. The first 0.5 mL of blood were discarded (catheter dead space was 0.3 mL) and the next 2 mL were collected in a heparinized tube. Plasma was obtained by centrifugation at 3000 g for 15 min and then stored at –20°C until used.

After thawing at room temperature, plasma samples were homogenized and centrifuged before solid-liquid extraction. Aliquots of 500 μL were acidified with 10 μL of phosphoric acid. An aliquot (300 μL) of the acidified sample was applied to C 18 precolumns (LiChrolut, Merck) previously prepared with 1 mL of methanol and 1 mL of 1% phosphoric acid. Columns were washed with 500 μL of methanol:water 10:90 (v/v) and 500 μL of hexane. SPF was eluted with 1 mL of methanol. The eluate was evaporated completely and the residue derivatized with L-leucinamide according to a method adapted from Foster and Jamali (1987).

Total bile output was collected during the following intervals after *rac*-SPF administration: 0–30 min, 30 min–l h, 1–2 h and 2–4 h. Samples were immediately acidified by the addition of 10 μL of phosphoric acid per mL of bile and frozen at –20°C until used. Urine samples (1 mL) were obtained by cistopuncture at 0, 30 min, 1, 2 and 4 h after *rac*-SPF administration. Samples were immediately acidified with 10 μL of phosphoric acid and frozen at –20°C until analysis.

After thawing at room temperature, bile and urine samples were vortexed and centrifuged. Enantiomers were extracted from an aliquot (150 μL) of bile and urine samples by acidification with 1N HCL and double extraction with acetone (5 mL), and dichloromethane (5 mL) respectively. In order to determine the total amount of SPF excreted as free and conjugated forms, another aliquot (150 μL) of each sample was treated with 1 M NaOH at 60°C for 30 min to induce an alkaline hydrolysis of the conjugates (putative

glucuronides) prior to the acidification (HCL) and extraction procedures.

After extraction, the residue was reconstituted in 60 µL of methanol and 20 µL were applied to silica gel thin layer chromatography (TLC) plates (Silica gel 60 F254 plates, Merck). The TLC plates were developed with a mixture of ethyl acetate and hexane (9:1, v/v). Spots on the TLC plates were detected under UV light, scrapped off and extracted with methanol. The methanol extract was evaporated completely and the residue was derivatized and injected into the HPLC system as described above.

The area under the plasma concentration–time curve (AUC) of SPF enantiomers up to the last plasma sampling time was determined using the linear trapezoidal method. For bile and urine samples, the difference between the AUC of hydrolysed and non-hydrolysed samples was assumed to correspond to the area of drug excreted as conjugates (putative glucuronides). The pharmacokinetics analysis was carried out using the PK Solutions 2.0
– Noncompartmental Pharmacokinetics Data Analysis program.

Differences between the pharmacokinetic parameters obtained from plasma, bile and urine for R (–) and S (+) SPF were evaluated using a Wilcoxon Signed Ranks Test (Conover, 1971). Values were reported as median and range. A P-value < 0.05 was considered statistically significant.

RESULTS

The arithmetic plot of the mean plasma concentrations of SPF enantiomers *vs* time after i.v. administration of the *rac*-SPF is shown in Fig. 1a. The plasma clearance was 739.5 (668.1) mL/h/kg for R (–) SPF and 783.3 (431.2) mL/h/kg for S (+) SPF. The AUC was 1.8 (1.2) µg/h/mL for R (–) SPF and 1.8 (0.7) for S (+) SPF. Half-life ($t_{1/2}$) was the same for R (–) as for S (+) SPF, 0.99 (1.0) h respectively. Secondary peaks were not found. For all the parameters described above, differences between enantiomers were not statistically significant.

The total amount of putative glucuronides excreted in the bile was 42.5 (16.1) µg/mL for R(–) glucuronide and 37.1 (26.5) µg/mL for S (+) glucuronide, representing 1% of the total dose administered (Fig. 3a). The total amount of SPF eliminated as free drug in bile and urine accounted for 0.3% and 0.12% of the total dose administered respectively. Only free SPF was found in the urine. Differences between SPF enantiomers for each matrix were not statistically significant.

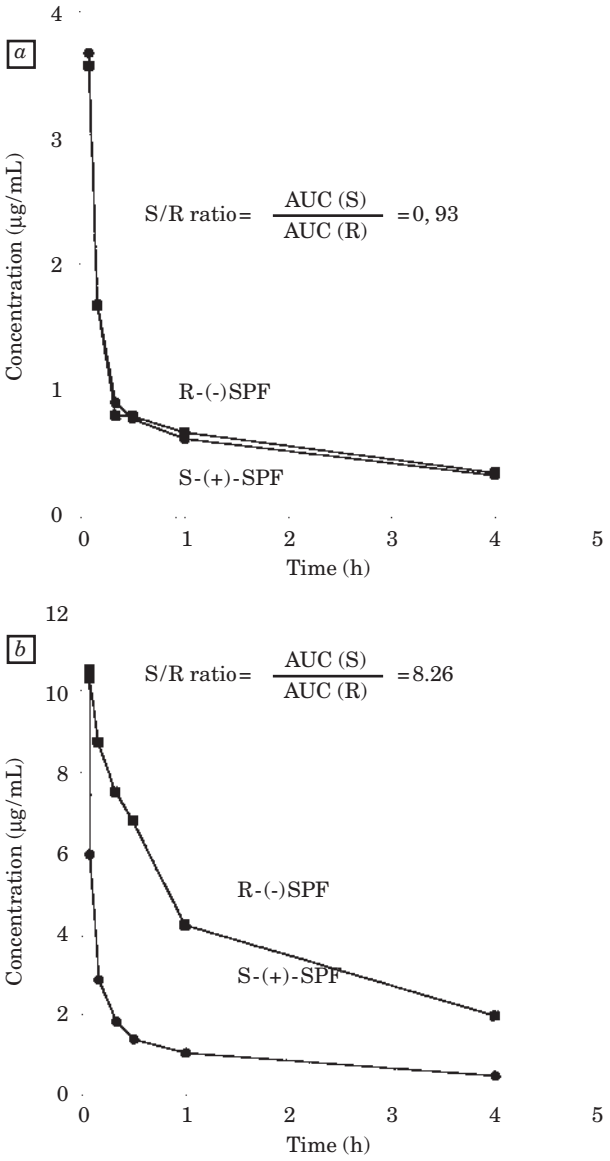

Fig. 1. Mean plasma enantiomeric ratios *vs.* time, and S/R ratios for the AUCs of suprofen (SPF) (a), and fenoprofen (FPF) (b), obtained after intravenous administration of the racemate in cats (50:50 each enantiomer). Reproduced with permission from Kluwer Academic Publishers. Castro *et al.* (1998).

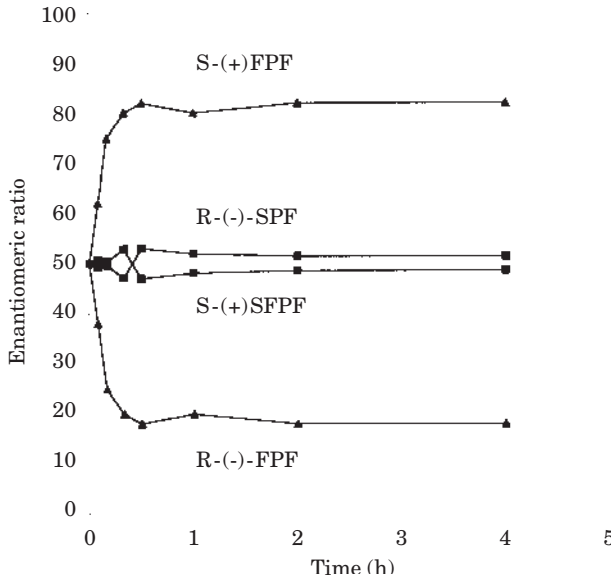

Fig. 2. Mean plasma enantiomeric ratios *vs.* time of SPF and FPF obtained after intravenous administration of the racemate in cats (50:50 each enantiomer).

DISCUSSION

A previous study has shown that SPF is poorly inverted in humans (Shinohara *et al.*, 1991). Other studies performed with *rac*-SPF in mice, guinea pigs, dogs and monkeys suggest the absence of metabolic chiral inversion (Mori *et al.*, 1984; Mori *et al.*, 1985). A similar pattern for the disposition of *rac*-SPF was observed in cats in the present study. Pharmacokinetic data obtained from plasma analysis suggest that the kinetic disposition of *rac*-SPF was not enantioselective. This is clearly seen after plotting the mean plasma concentrations (Fig. 1) and the enantiomeric ratios (Fig. 2) against time of *rac*-SPF and *rac* fenoprofen (FPF), which underwent a marked chiral inversion in the cat (Castro *et al.*, 1998). At the same i.v. doses of *rac*-SPF and *rac*-FPF, the S/R ratios for the AUC were 0.93 and 8.26 respectively (Castro *et al.*, 1998) (Fig. 1). In previous studies carried out with individual enantiomers of FPF, we have observed that the cat is capable of performing unidirectional (R) to (S) chiral inversion to a degree similar to that observed in the dog (Soraci *et al.*, 1996; Castro *et al.*, 2000). Therefore, the lack of SPF chiral inversion is not due to a metabolic characteristic of this species. Although the correct way to study the chiral inversion process for a particular compound is by the administration of each optically pure enantiomer individually, the marked similarity between the disposition of both SPF enantiomers suggests the absence of metabolic inversion (Delatour *et al.*, 1993). Our results would suggest that SPF is not a substrate of acylCoA ligase and, as a consequence, the formation of the R-suprofenyl CoA intermediate thioester that leads to chiral inversion would not occur.

Several studies *in vitro* have alluded to a possible multiplicity of xenobiotic CoA ligases to explain the differences in the thiosterification of profens (Knights, 1998). Recently, two isoforms of CoA ligase have been isolated from rat and human liver, however, only one of these appears to be the enzyme involved in the first step of the chiral inversion of the 2-arylpropionic acids (Sevoz *et al.*, 2000). If the same enzyme (from a catalytic point of view) is involved in the thiosterification of profens in different animal species, the structural constraints of the thiosterification substrate may vary from one compound to another (Soraci *et al.*, 1995). The conformational presentation of the substrate to the active site of the enzyme is one of the determinant factors of the thiosterification rate (Davankov, 1997). Thus, it is probable that in the cat, acyl CoA ligase recognizes R (–) FPF (Castro *et al.*, 2000) but not R (–) SPF.

In the present study, the biliary excretion of SPF enantiomers was not enantioselective. Carprofen (CPF), which belongs to the same NSAIDs group as SPF, is not inverted either by the dog or the cat (Taylor *et al.*, 1996). However, kinetic disposition of CPF is clearly enantioselective in the dog as a result of a preferential glucuronoconjugation of the S (+)-enantiomer (Delatour *et al.*, 1993; Soraci *et al.*, 1995; Priymenko *et al.*, 1998). In our study, the differences in biliary excretion of putative glucuronides between R (–) and S (+) SPF were not statistically significant ($P > 0.05$) (Fig. 3a). The same occurred with free SPF excretion in both bile and urine. Only free SPF, representing 0.12% of the administered *rac*-SPF dose, was recovered from the urine. The difference between urine enantiomer concentrations was not statistically significant ($P < 0.05$). Therefore, we hypothesize that the biological fate of SPF in the cat does not depend on the sequential physiological steps which could be potentially enantioselective (e.g., hepatic uptake, drug conjugation, bile canaliculi excretion or renal excretion of glucuronides) (Maire-Gauthier *et al.*, 1998; Priymenko *et al.*, 1998).

The contribution of the glucuronidation pathway to the *rac*-SPF detoxification was low (Fig. 3a)

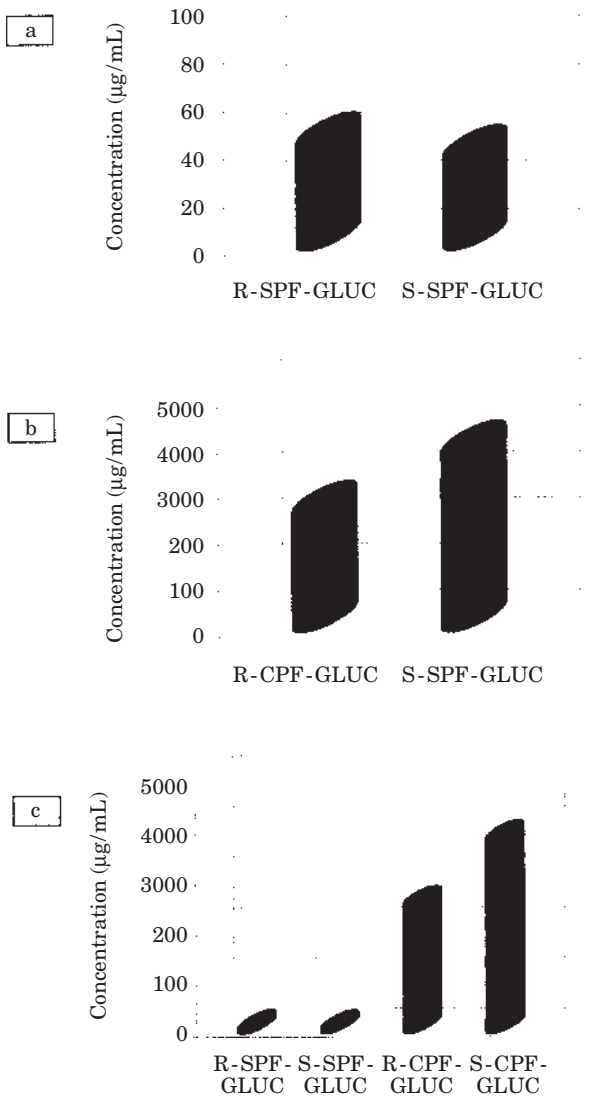

Fig. 3. Compared enantioselective glucuronidation (c), of suprofen in cats (a), and carprofen (CPF) in dogs (b), obtained after intravenous administration of the racemate (50:50 each enantiomer).
(GLUC : Glucuronides).

when compared with that of *rac*-CPF in the dog. The percentage of the total administered dose excreted in the bile in the present study was 1%, while for the dog, in the same period (4 h), the elimination of CPF glucuronide was about 10% (Soraci, 1995) (Figs 3b & c). This metabolic difference between cats and dogs may be due to the known difficulty of the domestic cat to conjugate certain xenobiotics with glucuronic acid (Baggot, 1977; Court & Greenblat, 1996; Court & Greenblat, 1997).

The metabolism of 2-arylpropionic acids involves three main pathways, (i) chiral inversion via formation of a reactive intermediate R (–) profen-CoA, (ii) glucuronidation and (iii) oxidation (Hutt & Caldwell, 1983; Voland *et al.*, 1990). Considering that neither chiral inversion nor glucuronidation seem to predominate in the cat, oxidation is likely to be the most important route for the metabolism of SPF enantiomers. In this regard, SPF was mainly metabolized by reduction of the ketone group to an alcohol in the dog, while in humans and rats hydroxylation of the thiophene ring was identified as the major pathway for SPF metabolism (Mori *et al.*, 1984; Mori *et al.*, 1985).

In conclusion, the disposition of *rac*-SPF in plasma was not enantioselective. This was probably due to the apparent lack of a significant unidirectional chiral inversion of R (–) SPF to S (+) SPF and the non-enantioselective glucuronidation and elimination of SPF in bile and urine. Our results also suggest that mechanisms other than the synthesis of SPF conjugates and the excretion of free SPF in bile and urine could account for most of SPF metabolism and disposition in cats. Further investigations should be carried out in order to determine the principal pathway(s) responsible for the plasma clearance of SPF. Finally, the results of this study reinforce the concept that enantioselective metabolism within a species can vary even for chemically similar compounds.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the contribution of E.M. Rodriguez to the data analysis.

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(Accepted for publication 24 February 2001, Published electronically 9 May 2001)