ENANTIOSELECTIVITY OF THE ENTEROHEPATIC RECYCLING OF CARPROFEN IN THE DOG

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ABSTRACT:
The disposition of the two enantiomers of carprofen (CPF), the (R)-CPF and the (S)-CPF, was investigated after iv administration of the racemate (4 mg/kg) in dogs equipped with a chronic bile duct catheter. Studies in dogs with diverted bile flow showed that both enantiomers were extensively excreted in bile with 74% of the (R)-enantiomer and 92% of the (S)-enantiomer from the iv administered dose being recovered in the bile as the respective glucuronide conjugates. The direct administration of acidic bile containing acyl-glucuronides of CPF in the duodenum showed that both conjugated enantiomers led to high CPF enantiomer systemic availability. However, comparison of CPF pharmacokinetics between dogs with nondiverted bile flow and dogs with diverted bile flow suggested that CPF was subjected to enantioselective enterohepatic recycling (EHC) and that only the (S)-CPF was recylced. The absence of EHC for the (R)-CPF is hypothesized to be the result of formation of glucuronidase-resistant isoglucuronides (epimers) to a greater extent for the (R)-CPF than for the (S)-CPF.

The objectives of the present study were (a) to demonstrate the existence of EHC for CPF in dogs and (b) to see whether one or several steps in this EHC were enantioselective.

Materials and Methods

Animal Preparation. Eleven male beagle dogs (10–15 kg body weight) were used, two for the validation of the surgical procedure and nine for the pharmacokinetic studies. All dogs were equipped with a biliary bypass catheter placed in the common bile duct. After a 24-hr fasting period, the surgical procedures were performed under halothane anesthesia 2.5% (v/v) with pentothal sodium induction (10 mg/kg, iv). When the common bile duct had been identified distally to the last hepatic duct, two 40-cm silicone rubber tubes (Sigma Medical, Nanterre, France; 1.5 mm i.d., 3.0 mm o.d.), prepared with a thiombarbital sodium induction (10 mg/kg, iv), were inserted until the duodenal lumen. The first catheter was inserted in the hepatic direction and the second toward the duodenum. They were anchored in position by a ligature behind the thickening. Both catheters were externalized through separate stab holes in the right flank and connected immediately. The catheters were protected with a specially constructed bandage. Antibiotics (penicillin, 200,000 units; dihydrostreptomycin, 0.2 g) were given daily by im injection for 5 days after surgery. The dogs were allowed to recover for 10 days after surgery. They were housed in metabolic cages and fed dried food (Royal Canin, France) 350 g per day containing 20% protein, 3.5% fiber, and 6.5% fat, mixed with 350 ml of water.

Drug Administration. rac-CPF (Zenecarp, C-vet Ltd., Bury St. Edmunds, UK) was administered iv via the radial vein at the dosage rate of 4 mg/kg. Two dogs received rac-CPF before and after surgery for validation of the surgical procedure. For the pharmacokinetic studies, nine dogs received rac-CPF on two separate occasions; i.e. with nondiverted or diverted bile flow.

CPF glucuronides [(R,S)-Gluc; i.e. (R)-Gluc and (S)-Gluc resulting from the glucuronidation of (R)-CPF and (S)-CPF, respectively] were administered iv via the radial vein or perfused for 10 min iv via the biliary catheter inserted in the duodenal direction. (R,S)-Gluc were obtained from pools of bile sampled from donor dogs. The bile was collected after iv administration of 4 mg/kg rac-CPF and immediately acidified with phosphoric acid (85%; Prolabo, France) (1/1000, v/v) to avoid acyl hydrolysis of the glucuronides. The id-administered dose, expressed as CPF equivalent, was 1.924 ± 0.183 mg/kg.

(±)-6-Chloro-α-methylcarbazole-2-acetic acid (carprofen, CPF1) is a nonsteroidal anti-inflammatory drug used in humans and dogs. CPF is an aryl propionic acid containing an asymmetrical carbon atom and existing in two enantiomeric forms, i.e. (R)-CPF and (S)-CPF. The racemic form of the drug (rac-CPF) is the one marketed.

It has been shown in dogs that no CPF chiral inversion [i.e. transformation of the (R)-CPF to the (S)-CPF] occurs in vivo (Benoit et al., 1993) or in vitro (Benoit et al., 1993; McKellar et al., 1994) and that the two enantiomers exhibit dissimilar disposition (McKellar et al., 1994; Delatour et al., 1993). There is no evidence in the literature of pharmacokinetic interactions between the (R)-CPF and the (S)-CPF.

CPF is excreted in the bile with 70% of an iv dose of CPF being eliminated in the feces, mainly as the glucuronide conjugate (Rubio et al., 1980). Like many other drugs displaying such excretion, CPF can be expected to undergo enterohepatic recycling (EHC) in dogs. EHC involves several physiological events such as hepatic uptake and conjugation of the drug, its biliary excretion (as drug or glucuronide) into the gut, intestinal hydrolysis of the glucuronide by microflora enzymes, and absorption of the free drug from the digestive tract into the portal circulation. Some of these steps, i.e. hepatic metabolism, biliary excretion, and glucuronide hydrolysis, might be stereoselective (Jamali et al., 1989; Lapicque et al., 1993; Sweeny and Nellans, 1992). CPF offers a unique advantage for demonstrating enantioselectivity in EHC because it does not undergo a chiral inversion in vivo.

1 Abbreviations used are: CPF, carprofen; EHC, enterohepatic recycling; rac-CPF, racemic form of (R,S)-CPF; MRT, mean residence time; ANOVA, analysis of variance.

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were applied on C18 precolumns (Vac Elut System, Varian, Les Ulis, France), phosphoric acid, 20:80 (v/v). For bile flow. The sampling times were 0, 2, 4, 8, 15, 30, and 60 min and 2, 4, 6, of administrations. No food was given during the study with the bile flow diverted and then daily for the other types of administrations.

**Blood Sample Collection.** Blood samples (2 ml) were taken from the jugular vein by direct puncture and collected for 72 hr in dogs with nondiverted bile flow. The sampling times were 0, 2, 4, 8, 15, 30, and 60 min and 2, 4, 6, 8, 12, 24, 36, 48, 60, and 72 hr after iv administrations of rac-CPF and iv or id administrations of (R,S)-Gluc. Blood samples (2 ml) were collected for 24 hr at 0, 2, 4, 8, 15, 30, and 60 min and 2, 4, 6, 8, 12, 16, 20, and 24 hr after iv administrations of rac-CPF in dogs with diverted bile flow. All blood samples were collected in heparinized tubes and centrifuged within 10 min. The plasma was immediately acidified with phosphoric acid (1/1000, v/v) to prevent hydrolysis and epimerization of the glucuronide conjugate and frozen at −20°C until analysis.

**Method of Bile Collection and Bile Restitution.** For the assay carried out in dogs with diverted bile flow, the gallbladder was emptied by opening the biliary circuit 5 min before the iv rac-CPF administration. The gallbladder catheter was then closed at the rac-CPF injection time to prevent bile flow. From this time until 24 hr after the rac-CPF administration, all the bile produced was collected by opening the catheter hourly, from 1 to 8 hr after the administration and then every 4 hr. The bile samples were collected in tubes protected against the light, immediately acidified (see above), and frozen at −20°C until analysis. Control bile (obtained from donor dogs) was administered into the catheter inserted in the distal common bile duct to compensate for the withdrawal of sampled test bile. Five or 10 ml of control bile was infused about 10 min after each collection of test bile. The restituation volume of control bile was fixed at 5 ml when the volume of collected bile was between 5 and 10 ml, and at 10 ml when the volume of collected test bile was more than 10 ml. The overall volume of control bile administered was 59% of the actually sampled test bile.

**Urinary Collection.** After iv rac-CPF injection in dogs with nondiverted bile flow, urine was collected by spontaneous voiding from one dog about every 4 hr until 72 hr after administration. The urine was collected in an opaque bag containing phosphoric acid as a glucuronide hydrolysis and epimerization inhibitor. Urine samples were measured, and aliquots were stored at −20°C until analysis.

**Experimental Design.** After surgery, the three different administrations (i.e. rac-CPF iv administration in dogs with nondiverted bile flow, rac-CPF iv administration in dogs with diverted bile flow, and id administration of bile containing (R,S)-Gluc in dogs with nondiverted bile flow) were carried out according to the design presented in table 1. Administrations were separated by a washout period of 1 week. Bile containing (R,S)-Gluc was administered iv in dogs with nondiverted bile flow after the completion of these three phases.

**Analytical Method: Sample Processing.** Plasma samples were thawed at room temperature and then vortexed and centrifuged. Aliquots (100 to 500 µl) were applied on C18 pre-columns (Vac Elut System, Varian, Les Ulis, France), which were subsequently processed differently depending on the analytes to be measured. For total (R)-CPF and individual glucuronides, the precolumns were washed with 500 µl of 1% phosphoric acid and 500 µl of methanol/1% phosphoric acid, 20:80 (v/v). For (R)-CPF and (S)-CPF, the precolumn was first washed with 1 ml of methanol/water 20:80 (v/v) and then eluted with 1 ml of methanol in a small vial. The eluate was evaporated to dryness and the residue derived according to the method of Spahn et al. (1988) using L-leucinamide hydrochloride (Fluka, St Quentin Fallavier, France) as deriving agent. The diastereoisomers thus produced were reappplied on the same pre-column.

Bile samples were centrifuged, diluted to 1/10 with 1% phosphoric acid and 20 µl injected on the column, allowing the direct quantification of (R)-CPF and (S)-CPF and individual glucuronides.

**Analytical Method: Sample Analysis.** Total (R,S)-CPF (i.e. (R)-CPF and (S)-CPF), (R)-CPF, (S)-CPF, (R,S)-Gluc, and (R,S)-CPF were quantified by HPLC. The apparatus consisted of an automated advanced sample processor (Varian, Les Ulis, France), two 110 B pumps (Beckman, Gagny, France), a 421A controller (Beckman), an RF 530 fluorometric detector (Shimadzu, by Tauras and Matignon, Paris, France) and a 3393 A integrator-calculator (Hewlett-Packard, Les Ulis, France). The column (250 × 4.6-mm internal diameter) was packed with a 5-µm RP18 Kromasil (Interchim, Montluçon, France). The binary mobile phases were gradients of acetonitrile (Merck, Nogent/Marne, France) (gradient A) and 20 mmol of Na2 HPO 4,2H 2 O and 10.7 min for (S)-Gluc. (R,S)-CPF (fig. 1A). The absolute extraction rate was 95%, and the linearity of both calibration curves was assessed up to 10 µg/ml. The resolution factor α was 3.5, and the limit of quantification was 0.15 µg/ml for each enantiomer. The repeatability was less than 6%.

For individual glucuronides and (R,S)-CPF, the gradient was 73% of gradient B for 6 min, and increasing to 60% within 10 min. The retention times were 14.2 min for (S)-Gluc, 15 min for (R)-Gluc, and 10.7 min for (R,S)-CPF (fig. 1A). The resolution factor α was 1.05, the absolute extraction rate from plasma was 85% (and 100% for bile), and the calibration curves were linear up to 10 and 1000 µg/ml for plasma and bile, respectively. The limit of quantification was 0.15 µg/ml in plasma, 1 µg/ml in bile, and 0.01 µg/ml in urine for each glucuronide. The repeatability was less than 6%. The glucuronide concentrations were expressed in CPF equivalents.

**Pharmacokinetic Analysis.** The plasma clearance (Cl) and the steady-state volume of distribution (Vss) were calculated using a noncompartmental approach (Gibaldi and Perrier, 1982). The AUC and the mean residence time (MRT) were calculated by the trapezoidal rule without extrapolation to infinity (Yamaoka et al., 1978).

The plasma clearance of each unconjugated CPF enantiomer (Cl(R) or Cl(S)) was obtained using eq. 1 or eq. 2, respectively.
The plasma clearance of each glucuronide (Cl_{(R)-Gluc}) and Cl_{(S)-Gluc}) was obtained using eq. 3 or eq. 4, respectively,

\[
Cl_{(R)-Gluc} = \frac{Dose_{(R)-Gluc}}{AUC_{(R)-Gluc}}
\]

(3)

\[
Cl_{(S)-Gluc} = \frac{Dose_{(S)-Gluc}}{AUC_{(S)-Gluc}}
\]

(4)

with Dose_{(R)-Gluc} and Dose_{(S)-Gluc} the iv doses of (R)-Gluc and (S)-Gluc (expressed in terms of free CPF); AUC_{(R)-Gluc} and AUC_{(S)-Gluc} the AUC of (R)-Gluc and (S)-Gluc following iv (R,S)-Gluc administration in dogs with nondiverted bile flow.

The system availability (F\%) of (R)-CPF and (S)-CPF after id administration of their respective glucuronide was obtained from dogs with nondiverted bile flow using eq. 5 or eq. 6, respectively (Shepard et al., 1985).

\[
F% = \frac{AUC_{(R)-Gluc}}{AUC_{(R)-Gluc}} \times \frac{\text{Dose}_{(R)-Gluc}}{\text{Dose}_{(R)-Gluc}} \times 100
\]

(5)

\[
F% = \frac{AUC_{(S)-Gluc}}{AUC_{(S)-Gluc}} \times \frac{\text{Dose}_{(S)-Gluc}}{\text{Dose}_{(S)-Gluc}} \times 100
\]

(6)

with Dose_{(R)-Gluc} and Dose_{(S)-Gluc} the id doses of (R)-Gluc and (S)-Gluc (expressed in terms of free CPF); AUC_{(R)-Gluc} and AUC_{(S)-Gluc} the AUC of (R)-Gluc and (S)-Gluc following id (R,S)-Gluc administration in dogs with nondiverted bile flow. AUC_{(R)-Gluc} after iv, AUC_{(S)-Gluc} after iv, Dose_{(R)-Gluc}, and Dose_{(S)-Gluc} are as for eq. 1 and eq. 2.

The estimated recirculating fraction during one cycle was calculated by the product of F as measured by the id administration of glucuronide and of F, the fraction of dose excreted in the bile directly measured in dogs with diverted bile flow.

The effect of the modalities of EHC was analyzed using an analysis of variance (ANOVA) to partition the variation into its potential sources (i.e. main effects: enantiomer and dog). Multiple comparisons were carried out using the Bonferroni approach whenever the ANOVA revealed heterogeneity between means. A one-way ANOVA with Bonferroni approach was applied for other analyses except when tests revealed variance heterogeneity. In these cases, nonparametric tests were carried out using Kruskal-Wallis analysis.

### Results

#### Influence of the Surgical Procedure on CPF Disposition.

The plasma AUC of (R,S)-CPF after iv administration of rac-CPF was similar before and after surgery in the two investigated dogs: 148 \(\mu g/ml\) vs. 145 \(\mu g/ml\) for one dog, and 203 \(\mu g/ml\) vs. 208 \(\mu g/ml\) for the other, respectively.

(R)-CPF and (S)-CPF Disposition in Dogs with Nondiverted Bile Flow. The semilogarithmic plot of the plasma concentrations (\(\mu g/ml\)) of CPF enantiomers vs. time (hr) after iv administration of rac-CPF in a representative dog with nondiverted bile flow is shown in fig. 2. The concentrations of (R)-CPF exceeded those of (S)-CPF at all sampling times. The plasma concentrations of (S)-CPF but not of (R)-CPF tended to increase at 8 hr; i.e. after meal time, leading to a plasma bump.

The plasma clearances were 17.1 \(\pm 4.0\) ml/hr/kg for (R)-CPF and 28.2 \(\pm 9.7\) ml/hr/kg for (S)-CPF (ANOVA, \(p < 0.01\)). The \(V_{ss}\) values were 0.12 \(\pm 0.03\) liter/kg for (R)-CPF and 0.19 \(\pm 0.05\) liter/kg for (S)-CPF (ANOVA, \(p < 0.01\)). The MRTs were 6.7 \(\pm 0.8\) hr and 7.1 \(\pm 1.0\) hr for (R)-CPF and (S)-CPF, respectively (ANOVA, \(p > 0.05\)). Individual kinetic parameters are given in table 2.

Cl, \(V_{ss}\), and MRT calculated from 0 to infinity differed by less than 15% from the values obtained over the first 24 hr, and so the 0–24-hr calculated parameters were retained for comparison with those subsequently obtained with diverted bile flow.
The plasma concentrations of glucuronides were very low after rac-CPF administration with nondiverted bile flow. They were quantifiable in seven and in two of the nine dogs for (S)-Gluc and (R)-Gluc, respectively (see individual values, table 3).

Urinary Excretion in One Dog with Nondiverted Bile Flow. Only glucuronides of CPF were recovered in the urine after an iv administration of rac-CPF, representing 0.01% for (R)-enantiomer and 0.08% for (S)-enantiomer of the administered rac-CPF dose.

(R)-CPF and (S)-CPF Disposition in Dogs with Diverted Bile Flow. The semilogarithmic plot of the plasma concentrations (µg/ml) of CPF enantiomers vs. time (hr) after iv administration of rac-CPF in a representative dog with diverted bile flow is shown in fig. 3. The concentrations of (R)-CPF always exceeded those of (S)-CPF, and the difference between the two enantiomers increased progressively with time. Unlike the dog with nondiverted bile flow (fig. 2), no secondary peak was observed for the (S)-CPF.

The plasma clearances were 16.1 ± 5.8 ml/hr/kg for (R)-CPF and 37.8 ± 14.2 ml/hr/kg for (S)-CPF (ANOVA, p < 0.01). Individual kinetic parameters are given in table 4. When these clearance values were compared with those obtained with nondiverted bile flow, it seemed that interrupting the EHC significantly increased the CI of (S)-CPF (+34%, ANOVA, p < 0.05) but did not modify significantly the CI of (R)-CPF (−6%, ANOVA, p > 0.05). According to eq. 11 and eq. 12, these percentages actually corresponded to the recirculated fractions, about 34% for the (S)-CPF and about 0% (−6%) for the (R)-CPF.

The Vm values were 0.11 ± 0.01 liter/kg for (R)-CPF and 0.17 ± 0.03 liter/kg for (S)-CPF (ANOVA, p < 0.01). No difference in Vm values was observed between nondiverted and diverted bile flow conditions for either enantiomer (ANOVA, p > 0.05).

The MRTs for dogs with diverted bile flow were 7.4 ± 1.4 hr and 5.2 ± 1.6 hr for (R)-CPF and (S)-CPF, respectively (ANOVA, p < 0.01). When these values were compared with those obtained from dogs with nondiverted bile flow, it seemed that interrupting the EHC significantly decreased the MRT of (S)-CPF (ANOVA, p < 0.01) but did not modify that of (R)-CPF (ANOVA, p > 0.05).

The plasma concentrations of glucuronides increased after rac-CPF administration in dogs with diverted bile flow and were quantifiable in every dog for (S)-Gluc and in all but two dogs for (R)-Gluc (see individual values, table 3).

Biliary Excretion of CPF Glucuronides after iv Administration of rac-CPF in Dogs with Diverted Bile Flow. Following an iv administration of rac-CPF, most of the CPF was eliminated in bile as CPF glucuronides, and less than 7% of the dose was excreted as free CPF. The mean amounts of (R,S)-Gluc excreted in the bile during the first 24 hr were 1483 ± 483 µg/kg for (R)-Gluc and 1842 ± 580 µg/kg for (S)-Gluc, representing 74 and 92% of the iv CPF dose administered, respectively. The CPF biliary clearances via the glucuronidation pathway calculated from eq. 9 and eq. 10 were 12.7 ± 8.4 ml/hr/kg for (R)-CPF and 36.8 ± 18.9 ml/hr/kg for (S)-CPF (ANOVA, p < 0.01).

Systemic Availability of CPF after iv Administration of (R,S)-Gluc in Dogs with Nondiverted Bile Flow. According to eq. 5 and eq. 6, the estimated absolute systemic availabilities of (R)-CPF and (S)-CPF from their respective glucuronides were 59.9 ± 29.4% for (R)-Gluc and 69.0 ± 38.8% for (S)-Gluc (Kruskal-Wallis analysis, p > 0.05). The individual kinetic parameters are given in table 5.

Using the results of this experiment, the estimated recirculating fraction during one cycle was 44% (0.74 × 59.9) for (R)-CPF and 63% (0.92 × 69.0) for (S)-CPF.

The plasma concentrations of (R)-Gluc and (S)-Gluc following iv administration of (R,S)-Gluc administration could not be quantified in four out of seven dogs for either glucuronide (see individual values, table 3).

Glucuronides Disposition after iv Administration of (R,S)-Gluc in Dogs with Nondiverted Bile Flow. The plasma clearances were 455 ± 325 ml/hr/kg for (R)-Gluc and 224 ± 177 ml/hr/kg for (S)-Gluc (Kruskal-Wallis analysis, p > 0.05). The plasma AUCs of free CPF were 36.2 ± 23.9 µg·hr/ml for (R)-CPF and 33.6 ± 16.1 µg·hr/ml for (S)-CPF. Using eq. 7 and eq. 8, the estimated percentages of glucuronides transformed back to the free moiety were 66.8 ± 32.1% for (R)-Gluc and 77.9 ± 22.6% for (S)-Gluc (Kruskal-Wallis analysis, p > 0.05). The individual kinetic parameters are given in table 6.

Discussion

This experiment showed that CPF undergoes an enantioselective EHC in dog, with only the (S)-enantiomer being significantly recyciol. The bile constitutes the major route of CPF excretion as the glucuronide conjugates, and both acyl-glucuronides are able to release highly bioavailable CPF in the intestine under acidic experimental conditions, but apparently not for the (R)-enantiomer under alkaline physiological conditions.

It was shown in dogs that about 25% of a labeled CPF dose was excreted in the bile within 5 hr of an iv CPF administration, but EHC was not demonstrated (Rubio et al., 1980). More recently, McKellar et al. (1990), using a nonchiral analytical method, hypothesized the existence of an EHC in dogs to explain a relatively large interanimal difference in plasma elimination half-life following iv CPF administration, although the plasma concentration profile did not support such a hypothesis.

A similar conclusion could be proposed for the present experiment if a nonchiral analytical method had been used. However, the use of a specific chiral analytical method permitted demonstration of a secondary rise in plasma concentrations for the (S)-CPF but never for the (R)-CPF in dogs with nondiverted bile flow. These results suggested the presence of an enantioselective EHC for CPF in dogs. This
was demonstrated by results obtained in dogs with diverted bile flow in which the plasma (R)-CPF clearance remained unmodified, whereas the plasma (S)-CPF clearance increased significantly. Furthermore, this experiment allowed calculation of the per cent of (S)-CPF recirculated at about 34% of the dose.

EHC is a complex phenomenon involving many sequential physiological steps, some of which are potentially subjected to stereoselectivity. These include CPF hepatic metabolism, active biliary excretion of glucuronides, and intramolecular rearrangement (“acyl-migration”) in the bile or small intestine of excreted acyl-glucuronides to form epimers; i.e. β-glucuronidase-resistant acyl glucuronide isomers.

The plasma clearances of (S)-CPF and (R)-CPF were measured in dogs with nondiverted or diverted bile flow. When clearance is measured in the first condition by integration of the time course of drug disposition in the blood circulation system but also the processes occurring in the intestinal tract, which is kinetically outside the body (Yamaoka et al., 1990). Thus, the plasma clearance obtained in the diverted bile flow condition must be considered as a measurement of the actual ability of the dog to eliminate CPF. Because the presence of a chronic bile duct catheter may compromise normal hepatic function (Wang and Reuning, 1994), we verified in two dogs that the CPF concentration-time profiles were similar before and after surgery. When the bile flow was diverted, an exogenous source of bile was infused to maintain physiological excretion of drug by the bile. Although we did not obtain any evidence with this approach that diverting the bile flow could have influenced CPF excretion, such a possibility cannot be excluded, as others have shown that an interruption of the normal bile flow may create some differences compared with physiological conditions (Ouellet and Pollack, 1995).

A large difference between plasma clearance of (R)-CPF and (S)-CPF was observed in the diverted bile flow condition, the clearance of (S)-CPF being more than twice that of (R)-CPF. The clearances of both (R)-CPF and (S)-CPF were low, representing less than 1% of the cardiac output, and of hepatic origin, as no renal elimination of CPF and almost total elimination of CPF by the bile were observed. Clearance of drugs with a low hepatic clearance is equal to the product of the unbound plasma fraction of the drug and its hepatic intrinsic clearance (Wilkinson and Shand, 1975). It can thus be assumed that the plasma clearance of CPF enantiomers can only be affected by stereoselectivity with regard to plasma binding and/or hepatic intrinsic clearance. The enantioselectivity of CPF plasma protein binding has been demonstrated in man, the S configuration exhibiting a higher affinity for human serum albumins than the R configuration (Iwakawa et al., 1990). Binding of CPF in dog is unknown, and further investigations will be necessary to explain the enantioselectivity of CPF plasma clearance, in terms of enantioselective plasma binding, enantioselective intrinsic hepatic clearance, or both.

The second physiologically based and independent pharmacokinetic parameter is the volume of distribution (V ss). In the present experiment, a significant difference between the two enantiomers was observed, whatever the bile flow condition (nondiverted or diverted) with (S)-CPF showing a larger V ss than (R)-CPF. The extent of physiological distribution of a drug is determined by the balance between its binding in plasma and in tissues (Wilkinson and Shand, 1975). It follows that (R)-CPF and (S)-CPF may differ in the relative extent to which they bind to plasma and tissue components. The higher clearance and V ss observed for (S)-CPF in the present experiment would be consistent with the hypothesis of a higher free fraction of (S)-CPF than of (R)-CPF in dog.

Little is known about the stereoselectivity of the active processes involved in the biliary secretion of drugs and their metabolites, although differences in the recovery of antipodes in the bile have been demonstrated (Forster et al., 1989; LeCorre et al., 1992). Direct estimates of biliary clearance are necessary when investigating the enantioselectivity of biliary excretion to eliminate the contribution of any other sources of stereoselectivity (Tucker and Lennard, 1990). In the present experiment, the biliary clearances of CPF enantiomers were measured throughout the glucuronidation process. The biliary clearance of (S)-CPF was three times higher than that of (R)-CPF. The origin of this enantioselectivity could be due to hepatic glucuronidation and/or to biliary glucuronide excretion through the bile canaliculi. The first mechanism has already been considered (see considerations about hepatic intrinsic clearance), and a higher production rate of the
(S)-Gluc is probably an important factor contributing to the observed difference in the biliary clearance between the two enantiomers. The excretion of a glucuronide conjugate in the bile requires its passage from the hepatocytes to the bile. Thus, the rate of transfer out of the parenchymal cells may influence the rate of excretion of glucuronide in bile. If this transfer operates against a concentration gradient (high parenchymal cells may influence the rate of excretion of glucuronide from the hepatocytes to the bile. Thus, the rate of transfer out of the bile, less than 7% of CPF occurring in the bile in the free form. In

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\text{Difference not significant (} p > 0.05; \text{ANOVA, Kruskal-Wallis).}
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\text{\textbf{TABLE 4}}
\]

Pharmacokinetic parameters (mean ± SD) of (R)-CPF and (S)-CPF after iv administration of rac-CPF (4 mg/kg) in dogs with diverted bile flow

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<tr>
<th>Parameters(^a) (units)</th>
<th>Dogs</th>
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<tr>
<td>CI (ml/hr/kg)</td>
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<tr>
<td>R</td>
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<td>S</td>
<td>27.1</td>
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<td>CI(_b) (ml/hr/kg)</td>
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<tr>
<td>R</td>
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<tr>
<td>S</td>
<td>17.6</td>
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<tr>
<td>V(_{SS}) (liter/kg)</td>
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<tr>
<td>R</td>
<td>0.12</td>
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<tr>
<td>S</td>
<td>0.18</td>
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<tr>
<td>MRT (hr)</td>
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<tr>
<td>R</td>
<td>8.7</td>
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<td>S</td>
<td>6.5</td>
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\(^a\) Values were obtained by the trapezoidal rule without extrapolation to infinity (0–24 hr).
\(^b\) Statistically significant difference (} p < 0.01; ANOVA, Bonferroni) between (R)-CPF and (S)-CPF.

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\text{\textbf{TABLE 5}}
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Pharmacokinetic parameters (mean ± SD) of (R)-CPF and (S)-CPF after id administration of bile containing glucuronides of CPF (1.924 ± 0.183 mg/kg of (R)-Gluc, 2.523 ± 0.129 mg/kg of (S)-Gluc with 0.289 ± 0.009 mg/kg as free CPF), in dogs with nondiverted bile flow

<table>
<thead>
<tr>
<th>Parameters(^a) (units)</th>
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<td>AUC (µg·hr/ml)</td>
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<td>R</td>
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<td>F (%)</td>
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\(^a\) Values were obtained by the trapezoidal rule without extrapolation to infinity (0–72 hr).
\(^b\) —, not measured.
\(^c\) Difference not significant (} p > 0.05; ANOVA, Kruskal-Wallis).

\[
\text{\textbf{TABLE 6}}
\]

Pharmacokinetic parameters (mean ± SD) of (R)-Gluc and (S)-Gluc after iv administration of bile containing glucuronides of CPF (0.765 ± 0.096 mg/kg of (R)-Gluc, 0.975 ± 0.125 mg/kg of (S)-Gluc, with 0.195 ± 0.02 mg/kg as free CPF), in dogs with nondiverted bile flow

<table>
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<tr>
<th>Parameters(^a) (units)</th>
<th>Dogs</th>
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<tr>
<td>CI (ml/hr/kg)</td>
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<td>R</td>
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<td>f(_m) (%)</td>
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\(^a\) Values were obtained by the trapezoidal rule without extrapolation to infinity (0–72 hr).
\(^b\) —, not measured.
\(^c\) Difference not significant (} p > 0.05; ANOVA, Kruskal-Wallis).
the metabolites were then transported through the bile canaliculi (vide supra) while a fraction crossed the sinusoidal membrane to gain access to the systemic circulation. Because of analytical limitations, we were unable to measure the amount of glucuronide discharged directly in the plasma. We showed by the direct iv injection of bile glucuronides that CPF glucuronides were very rapidly cleared, as previously reported for naproxen glucuronide in rats (Iwaki et al., 1995). Almost all the administered dose of glucuronides was hydrolyzed back to CPF. The high plasma clearance of glucuronides was explained by the instability of the 1-β-ester linkage under physiological conditions, enzymatic or nonenzymatic hydrolytic cleavage regenerating the parent drug. This immediately reversible process has been reported as futile recycling (Meffin et al., 1983). This is why the direct plasma elimination of glucuronides is of no relevance in the overall CPF clearance.

In conclusion, the present study demonstrates that, in the dog, only the (S)-CPF is recycled under physiological conditions. We tentatively hypothesize that, although (R)-Gluc is largely excreted in bile, acyl-migration of 1-O-glucuronide in the small intestine under normal alkaline conditions prevents glucuronide hydrolysis and the subsequent reabsorption and enterohepatic recycling of the (R)-CPF. From a therapeutic point of view, our results suggest the possible enantioselective alteration of EHC by several conditions, including hepatic insufficiency (work in progress) and intestinal pH. Intestinal pH might alter the EHC of CPF, as the relative alkaline physiological environment of the intestine seems to ensure low availability of CPF from its hepatic-formed glucuronides, whereas an acidic environment seems to prevent glucuronide epimerization, which then allows glucuronide hydrolysis by the ileal microflora, which increases the systemic availability of CPF.

### References


