BILIARY EXCRETION AND ENTEROHEPATIC CYCLING OF R- AND S-FLURBIPROFEN IN THE RAT

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ABSTRACT:
According to a previously published report, R- and S-flurbiprofen glucuronides were excreted in the bile after iv administration of the pure enantiomers, but only R-flurbiprofen seemed to undergo enterohepatic cycling. To study the possible stereospecificity in the enterohepatic cycling of flurbiprofen (FL), we investigated the pharmacokinetics of R- and S-FL in control and bile-duct cannulated rats after iv administration of racemic FL (20 mg·kg⁻¹). FL pharmacokinetics were highly stereospecific in control rats: plasma clearance (CL) was much higher and distribution volume (Vd) larger for R-FL (2.60 ± 0.51 ml·min⁻¹·kg⁻¹ and 500 ± 59 ml·kg⁻¹, respectively) as compared with S-FL (CL: 0.72 ± 0.10 ml·min⁻¹·kg⁻¹, Vd: 312 ± 12 ml·kg⁻¹). Renal excretion of the R- and S-FL glucuronides was extremely small (<0.5%), whereas biliary excretion accounted for 8.3 ± 1.8% (R-FL glucuronide) and 14.3 ± 2.4% (S-FL glucuronide) of the administered dose. Bile-duct cannulation significantly increased CL of S-FL (0.90 ± 0.10 ml·min⁻¹·kg⁻¹ compared with 0.72 ± 0.10 ml·min⁻¹·kg⁻¹ in control rats, p <0.05), whereas CL of R-FL was not affected. Paired rat experiments in which the bile of the first rat was deviated into the duodenum of the second rat demonstrated measurable plasma concentrations of R- and S-FL in the receiver rat after iv administration of 20 mg·kg⁻¹ R, S-FL to the donor rat. Our results clearly show that R- and S-FL glucuronides are excreted via the bile and subsequently undergo hydrolysis followed by reabsorption of both R- and S-FL.

Flurbiprofen, a 2-arylpropionic acid (2-APA) nonsteroidal anti-inflammatory drug, is eliminated in the rat mainly by metabolism to several phase I metabolites and, to a smaller extent, by direct glucuronidation (1). Although unidirectional chiral inversion of R- to S-flurbiprofen is low in the rat (10–15%) and nonexistent in man, its pharmacokinetic behavior in both species demonstrates pronounced enantioselectivity (2). While studying the pharmacokinetics of the flurbiprofen enantiomers in the rat using iv microdialysis sampling, we consistently found a secondary peak in the unbound blood concentration-time profile of the S-enantiomer occurring between 2 and 4 hr after iv administration of racemic flurbiprofen (unpublished results, see fig. 1). Such a secondary peak was missing or was much smaller in the case of R-flurbiprofen. Assuming that this secondary peak is a result of enterohepatic cycling, these observations, which are not consistent with the results of a recent study suggesting that only R-flurbiprofen undergoes enterohepatic cycling in the rat (3), prompted us to investigate the enantioselective aspects of the biliary excretion of flurbiprofen and/or its glucuronide conjugate and its subsequent enterohepatic cycling in the rat.

Materials and Methods

Chemicals and Reagents. Racemic flurbiprofen and S-naproxen were purchased from Sigma Chemical Co. (St. Louis, MO). Pure R- and S-flurbiprofen enantiomers were kindly supplied by Dr. J. E. Jeffery, Boots Pharmaceuticals (Nottingham, UK). Solvents were of HPLC grade and all other chemicals used were AR grade.

Animals. Male Wistar rats (265–289 g) were housed in an environmentally controlled room at 20–22°C with a 12 hr light/dark cycle. Food (type A04, U.A.R., Epinay-sur-Orge, France) and tap water were provided ad libitum.

Bile Duct-Cannulated vs. Control Rats. Rats were anesthetized with a mixture of 4 mg/kg droperidol and 0.08 mg/kg fentanyl (Thalamonal, Janssen Pharmaceutica, Beerse, Belgium). Cannulae of silastic tubing (0.94 mm OD, 0.51 mm ID, Dow Corning, Valbonne, France) were implanted in the right and left jugular vein (control experiments). In some rats (bile-duct cannulated experiments), two additional cannulae were placed: one in the bile duct (PE-50 tubing) and one in the upper part of the small intestine (medical grade silicone tubing, 0.94 mm OD, 0.51 mm ID). All cannulae were exteriorized at the back of the neck. The control rats were sham operated, but no cannulae were introduced in the bile duct or the duodenum. The animals were allowed to recover from the surgery overnight. During recovery, the bile outflow was connected to the duodenal cannula in the bile duct-cannulated rats to prevent excessive loss of bile salts.

FIG. 1. Blood microdialysate concentrations of R-flurbiprofen (□) and S-flurbiprofen (■) in a single rat after iv administration of racemic flurbiprofen (20 mg/kg).

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Just before iv injection of racemic flurbiprofen (20 mg/kg), the bile duct cannula was disconnected from the duodenal cannula, and the animal was placed in a metabolic cage. Blood samples (250 μl) were collected in syringes containing heparin at the following times: 0 (blank), 5, 15, 30, 60, 120, 180, 240, 300, and 360 min. Total urine and total bile output were collected (in flasks on ice) during the following intervals: 0–4, 4–8, 8–24 h, and 0–2, 2–4, 4–6, 6–8, 8–24 h, respectively. Blank urine and bile were obtained just before flurbiprofen administration.

**Paired Rat Experiments.** Rats were surgically prepared as for the bile duct cannulation experiments with the exception that only one jugular cannula was implanted. The day after surgical implantation of the cannulae, the bile duct cannula of one rat (donor) was connected to the duodenal cannula of a second rat (receiver). Racemic flurbiprofen (20 mg/kg) was injected iv to the donor rat and serial blood samples (250 μl) were obtained from the receiver rat at the following times: 0 (blank), 30, 60, 120, 180, 240, 300, 360, 480 and 720 min.

**Non-Chiral HPLC Assays.** Concentrations of flurbiprofen in plasma, bile, and urine were determined before and after alkaline hydrolysis (5 N NaOH, 30 min, room temperature) using an HPLC method described before (4, 5) but with adaptations. Plasma, bile, and urine samples were diluted with methanol containing the internal standard (naproxen). After vortex mixing and centrifugation, an aliquot of the supernatant was injected onto the HPLC column. The eluate was monitored by UV detection at 254 nm (plasma) or by fluorometric detection (bile, urine) at the following excitation and emission wavelengths: 258 nm, 310 nm for flurbiprofen and 230 nm, 352 nm for naproxen (internal standard). Minimal quantifiable concentrations in plasma, bile, and urine were the following: 0.25 μg/ml, 1.0 μg/ml, and 0.1 μg/ml, respectively. Unlike bile which showed significant increases in flurbiprofen concentrations after alkaline treatment (owing to alkaline hydrolysis of the acyl glucuronide of flurbiprofen), plasma samples did not reveal the presence of quantifiable concentrations of flurbiprofen glucuronide. Biliary excretion of unchanged flurbiprofen was small (less than 1% of the administered dose) and was most likely a result of hydrolysis of flurbiprofen acyl glucuronide during sample collection and storage (6). In urine quantifiable flurbiprofen concentrations were only found after alkaline hydrolysis.

**Stereospecific HPLC Assay.** The mobile phase corresponding to the flurbiprofen peak eluting from the C\textsubscript{18} column was collected for each of the nonchiral HPLC methods described above. After evaporation, the residue was dissolved in 150 μl of the mobile phase and an aliquot (20 μl) was injected onto a CHIRAL-AGP column (5 μm, 100 × 4 mm, ChromTech AB, Hägersten, Sweden) to determine the R/S ratio. The mobile phase consisted of 91:9 (v/v) mixture of 20 mM potassium phosphate buffer (pH 6.5)/isopropanol containing 1 mM dimethyloctylamine. The analytical column was protected by a CHIRAL-AGP guard column (10 × 3 mm). Flow rate was 1 ml/min, and the eluate was monitored using a fluorescence detector (Spectrastream SL2000, Spectrastysics, San Jose, CA) using excitation and emission wavelengths at 258 and 310 nm, respectively. Column temperature was maintained at 12°C using a refrigerated circulator (CoolFlow CRT-33, Neslab, Newington, NH). The R- and S-flurbiprofen peaks showed baseline resolution and eluted at 5.0 and 7.5 min, respectively. The R/S ratio was calculated as the area under the curve of the R-flurbiprofen peak divided by the area under the S-flurbiprofen peak. Racemic flurbiprofen concentration determined by nonchiral HPLC methods was then converted to R- and S-flurbiprofen concentrations using the R/S ratio.

**Data Analysis.** Pharmacokinetic parameters were determined by the so-called “noncompartmental” approach. AUC values were calculated using the linear trapezoidal rule from 0 to t (last blood sampling time) with extrapolation to infinity (plasma concentration at time t divided by slope α\textsubscript{t}). Terminal plasma half-life (t\textsubscript{1/2t}) was calculated as 0.693/α\textsubscript{t}, where α\textsubscript{t} is estimated by linear regression of the terminal log-linear phase of the plasma concentration-time curve. Plasma clearance (CL\textsubscript{p}) was calculated as dose/AUC and apparent volume of distribution as CL\textsubscript{p}/α\textsubscript{t}. The fraction of the iv administered dose excreted in urine (f\textsubscript{u,gluc}) or bile (f\textsubscript{b,gluc}) as the glucuronide conjugate was calculated as the total amount of flurbiprofen glucuronide (expressed as flurbiprofen equivalents) recovered in urine or bile divided by the iv flurbiprofen dose. The clearance associated with the formation of the glucuronide conjugate CL\textsubscript{m,gluc} was calculated as (CL\textsubscript{um,gluc} × f\textsubscript{u,gluc}, where f\textsubscript{u,gluc} represents the fraction of the iv dose converted to glucuronide conjugate, i.e., the sum of f\textsubscript{r,gluc} and f\textsubscript{b,gluc}.

Comparisons between groups were performed by the paired or unpaired Student t test. A p-value of 0.05 or less was considered significant.

**Results and Discussion**

Pharmacokinetic parameters of R- and S-flurbiprofen obtained in the present study (fig. 2, table 1) are consistent with values reported previously (2, 7–9). They demonstrate that the disposition kinetics of flurbiprofen exhibit pronounced stereoselectivity in the rat. Studies have shown that this stereoselectivity is only marginally influenced by unidirectional chiral inversion of the R- to S-enantiomer (2, 7, 10). Stereoselectivity in metabolism and plasma protein binding of flurbiprofen have been suggested to account for these pronounced differences in the pharmacokinetic behavior of the R- and S-enantiomer (4, 5, 11).

Differences between enantiomers in biliary excretion and enterohepatic circulation can also contribute to stereoselectivity in drug disposition as has been shown in rats for carprofen, another 2-APA compound (12). Biliary excretion of flurbiprofen glucuronides has been demonstrated in rats (2, 3). In the present study, approximately 20% of the administered dose was recovered in bile as the sum of both
flurbiprofen glucuronides, whereas their urinary excretion was very small (ca. 0.25% of the administered dose). It is therefore not surprising that exteriorization of the bile duct only lead to relatively minor (although sometimes statistically significant) changes in the pharmacokinetics of the flurbiprofen enantiomers (fig. 2, table 1). The plasma clearance of S-flurbiprofen was higher (p < 0.05) in bile duct-cannulated rats (90 ± 0.10 ml/min kg⁻¹) compared with control rats (0.72 ± 0.10 ml/min kg⁻¹). A significant difference was also found between the biliary excretion of the R-flurbiprofen glucuronide (8.3 ± 1.8% of the administered dose) compared to S-flurbiprofen glucuronide (14.3 ± 2.4%). Partial metabolic clearances owing to the formation of the R- and S-flurbiprofen glucuronide were significantly different: 0.21 ± 0.04 ml/min kg⁻¹ vs. 0.10 ± 0.03 ml/min kg⁻¹, respectively. This indicates that the glucuronidation of flurbiprofen is stereospecific in the rat, a finding consistent with results obtained in vitro by using rat liver microsomes (5).

Based on calculations of the fraction of each flurbiprofen enantiomer undergoing enterohepatic circulation, Menzel et al. (3) concluded that R-flurbiprofen seemed to be almost quantitatively reabsorbed from the intestine, whereas the S-enantiomer seemed not to be reabsorbed. Our results do not support this conclusion, R- and S-flurbiprofen concentrations could be determined in plasma of the receiver rats during the paired rat experiments (fig. 3). The mean AUC of R- and S-flurbiprofen from 0 to 12 hr in the receiver rats of the paired rat experiments (N = 3) after iv administration of racemic flurbiprofen to the donor rats was 16.6 ± 9.9 μg h ml⁻¹ and 53.2 ± 22.1 μg h ml⁻¹, respectively. These results are direct proof that both enantiomers were reabsorbed from the gastrointestinal tract after biliary excretion of their glucuronide conjugates. Much higher plasma concentrations of S-flurbiprofen compared with R-flurbiprofen could be demonstrated in the receiver rats for two obvious reasons: 1) biliary excretion of S-flurbiprofen glucuronide (14.3%) is higher as compared with R-flurbiprofen (8.3%), 2) plasma clearance of R-flurbiprofen is higher as compared with the S-enantiomer, and 3) its Vd is larger compared with the S-enantiomer. Consequently, only the blood microdialysate concentration-time profiles of the S-enantiomer showed a clear secondary peak at approximately 3 hr after iv administration of racemic flurbiprofen (fig. 1). It is clear that the classical blood sampling schedules do not possess the necessary resolution to consistently reveal these secondary peaks in the plasma concentration-time profiles of S-flurbiprofen (fig. 2).

**References**