Stereoselective Disposition of Ibuprofen Enantiomers in the Isolated Perfused Rat Kidney

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The renal clearance of ibuprofen enantiomer was studied separately in the isolated perfused rat kidney at initial perfusate concentrations of 10 μg/ml (n = 4) and 100 μg/ml (n = 4). Perfusate and urine samples were measured for R(−) and S(+)-ibuprofen using a stereospecific HPLC assay; urine samples were also analyzed after alkaline hydrolysis. Functional viability of the kidney was assured by determining the fractional excretion of glucose and glomerular filtration rate (GFR) at similar perfusion pressures. The clearance of ibuprofen was equivalent to the apparent formation clearance of conjugated enantiomer since unchanged ibuprofen could not be detected in the urine. At 10 and 100 μg/ml, the clearance (±SD) of $R(-)$-ibuprofen was 2.50 ± 1.28 and 2.19 ± 1.42 μl/min, respectively. At 100 μg/ml, the clearance of $S(+)$-ibuprofen was 0.805 ± 0.290 μl/min. The protein binding of ibuprofen was found to be concentration dependent and favored the $R(-)$-enantiomer. The excretion ratio (clearance corrected for free fraction and GFR) of $R(-)$-ibuprofen was 0.398 ± 0.209 and 0.295 ± 0.209 for perfusate concentrations of 10 and 100 μg/ml, respectively. The excretion ratio of $S(+)$-ibuprofen was 0.0886 ± 0.0335 for perfusate concentrations of 100 μg/ml. These results demonstrate that the sum of renal mechanisms involved for the clearance of $R(-)$- and $S(+)$-ibuprofen was net reabsorption. Ibuprofen was recovered in the urine solely as conjugated material and no evidence of $R(-)$ to $S(+)$ conversion was observed. In addition, the data suggest that $R(-)$-ibuprofen is cleared through the kidney faster than its $S(+)$-enantiomer.

KEY WORDS: ibuprofen enantiomers; renal clearance; renal metabolism; rat isolated perfused kidney.

INTRODUCTION

Ibuprofen is a nonsteroidal antiinflammatory drug (NSAID) that is currently used for arthritic conditions in addition to its analgesic and antipyretic actions. Although detailed information is available concerning the pharmacokinetics of ibuprofen and other NSAIDS (1), only recently have the stereochemical disposition of these agents been addressed (2–5). This is of therapeutic importance for several reasons. First, ibuprofen is administered as racemate even though the drug's pharmacologic activity has clearly been shown to depend upon the $S(+)$-enantiomer. Second, chiral inversion of the $R(-)$- to the $S(+)$-isomer occurs to a significant extent. Third, stereoselective protein binding of ibuprofen enantiomers may complicate the interpretation of plasma concentrations. And fourth, under certain pathophysiological conditions, the long-term use of NSAIDs can lead to renal ischemia and acute renal failure.

Given the stereoselective disposition of ibuprofen along with its potential for renal toxicity, it is important to study the excretory and metabolic profiles of $R(-)$- and $S(+)$-enantiomers in the kidney. Therefore, the following objectives are proposed: (i) to define the renal clearance of $R(-)$-ibuprofen and $S(+)$-ibuprofen, (ii) to determine if ibuprofen enantiomers are metabolized in the kidney, and (iii) to determine if chiral inversion of ibuprofen enantiomers occurs in the kidney.

Studies were performed using an isolated, perfused rat kidney preparation (rat IPK). This allows for better control of $R(-)$-ibuprofen and $S(+)$-ibuprofen concentrations to be presented to the kidney and the absence of nonrenal factors that may influence *in vivo* drug disposition. In addition, one can directly and precisely measure the functionality of the rat IPK with respect to each enantiomer.

METHODS AND MATERIALS

Perfusate

The initial perfusate volume was 100 ml. It consisted of Krebs–Henseleit bicarbonate (KHB) buffer containing 6.00% bovine serum albumin (BSA) (fraction V; ICN ImmunoBiologicals, Lisle, IL), glucose (0.1%), and eight L-amino acids (Sigma Chemical Co., St. Louis, MO) (6). The BSA was previously dialyzed against an approximate five-fold excess of buffer without albumin (three changes over 48 hr at 4°C with shaking). The perfusing medium was aerated with humidified O_2:CO_2 (95:5) as it passed through a multibulb glass oxygenator and back into the glass reservoir; oxygenation occurred for ≥1 hr prior to arterial cannulation and throughout the length of the experiment. Perfusate pH was monitored with a Φ61 pH meter (Beckman Instruments, Inc., Fullerton, CA) and adjusted, if necessary, to 7.4.

Surgical Procedure

The rat IPK experiments were modeled after the methods described by Nishiitsutsuji-Uwo *et al.* (7) and Bowman (8). Male Sprague–Dawley rats (327–413 g) were anesthetized intraperitoneally with sodium pentobarbital (50 mg/kg body weight). The left superficial femoral vein was exposed and 100 mg of mannitol and 200 units of heparin were administered. A midline incision was made and the major abdominal vessels were isolated. A ligature was passed around the right renal artery, and proximal and distal ligatures were placed around the mesenteric artery. The right ureter was catheterized with PE-10 polyethylene tubing. The right renal artery was cannulated via the mesenteric artery and the hemostat holding back the perfusate was released upon entering the renal artery. The whole kidney was then excised, trimmed of adhering tissue, and transferred immediately to a

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recirculating perfusion apparatus, enclosed in a temperature-controlled (37°C) Plexiglas chamber. Perfusion pressure in the renal artery was controlled by monitoring the manometer and adjusting the flow-pressure valve accordingly. A correction was made for the intrinsic apparatus pressure.

Experimental Design

R(−)-Ibuprofen or *S*(+)-ibuprofen (3.08 mg/ml) was dissolved in KHB buffer with the aid of 4 *N* NaOH, and [^{14}C]inulin (16.7 μCi/ml; sp act, 2.0 μCi/mg; ICN Radiochemicals, Irvine, CA) was dissolved in distilled water. After a 15-min equilibration period, 0.325 or 3.25 ml of drug and 0.15 ml of inulin were introduced as a bolus into the recirculating perfusate; initial perfusate concentrations of each enantiomer were 10 μg/ml (n = 4) or 100 μg/ml (n = 4). An additional 15 min was then allowed for drug distribution to occur. The subsequent time (80 to 100 min) was divided into 10-min urine collection periods for the measurement of kidney function and drug disposition parameters. The urine volume was measured with a tuberculin syringe and the pH was determined immediately. Perfusate (1.5 ml) was sampled at the midpoint of each urine collection. Isovolumetric replacement of urine loss with buffer and perfusate sampling loss with blank perfusate was performed in order to minimize changes in perfusate composition during the experiment. Due to the instability of acyl glucuronides (9,10), all perfusate and urine samples were adjusted immediately to pH values between 2.0 and 3.5 with phosphoric acid and then frozen at −20°C until subsequent analysis.

Functionality of the rat IPK was assessed primarily by measuring glomerular filtration rate (GFR) and the fractional excretion of glucose (FEglucose). The clearance of inulin was taken to represent GFR. Clearances (CL_r) were calculated for ibuprofen enantiomers and inulin by dividing the urinary excretion rate of the substance by its perfusate concentration at the midpoint time interval.

Analytical

Perfusate and urine samples containing *R*(−)- and *S*(+)-ibuprofen were analyzed by adopting the stereospecific HPLC assay of Mehvar *et al.* (11). Urine was assayed before and after alkaline hydrolysis, and the difference was taken to represent conjugated ibuprofen. The hydroxy and carboxy metabolites of ibuprofen were sought in selected perfusate and urine (before and after alkaline hydrolysis) samples using a gradient HPLC assay (12). Radioactive measurements for [^{14}C]inulin were performed on an LS 3801 liquid scintillation counter (Beckman Instruments, Fullerton, CA) using an external standard method for quench correction. Glucose was determined with a YSI Model 27 Industrial Analyzer (Fisher Scientific, Chicago, IL) which utilizes an immobilized enzyme membrane mounted on the end of an electrochemical sensor.

Protein Binding

The protein binding of *R*(−)- and *S*(+)-ibuprofen was determined by spiking the blank perfusate with enantiomer over a concentration range of 20 to 500 μg/ml (run in triplicate). Perfusate (0.75 ml) was dialyzed against an equal volume of isotonic phosphate buffer (0.067 *M*, pH 7.4) in a Dubnoff Metabolic Shaking Incubator (VWR Scientific, Chicago, IL) at 37°C for 8 hr using Spectrapor 2 membrane tubing (Spectrum Medical Industries, Los Angeles, CA). Preliminary studies indicated that equilibrium was reached for ibuprofen within 6 hr and remained constant over 24 hr. Drug content in the dialyzed perfusate and buffer was then assayed by HPLC (12).

Ibuprofen has been shown to exhibit a nonlinear protein binding (13), and as a result, values for percentage free were determined accordingly (14,15). The volume-corrected bound (C_b'') and free (C_f') equilibrium concentrations of ibuprofen enantiomer were best fitted to a protein binding model which incorporates a single Langmuir term plus a linear term:

$$C_b'' = P1 \cdot C_f'/(P2 + C_f') + P3 \cdot C_f' \quad (1)$$

$P1$, $P2$, and $P3$ are the binding parameters that were obtained using the nonlinear least-squares regression program MINSQ (MicroMath Scientific Software, Salt Lake City, UT, 1988) and a weighting factor of unity. The quality of the fit was determined by evaluating the coefficient of determination (r^2) and the standard error of parameter estimates and by visually inspecting residuals.

Values for C_b'' were determined using the following equation:

$$C_b'' = V_p' \cdot (C_p' - C_f')/V_p \quad (2)$$

V_p and V_p' are the perfusate volumes before and after dialysis, respectively, C_p' is the measured total perfusate concentration after dialysis, and C_f' is the measured free concentration in buffer after dialysis.

Once the binding parameters ($P1$, $P2$, and $P3$) are known, the free concentrations of ibuprofen enantiomer in the original perfusate sample (C_f) can be determined by finding the positive root of the quadratic equation below for a given value of C_p, the measured total concentration of drug in recirculated perfusate prior to dialysis.

$$(1 + P3) \cdot Cf^2 + (P1 + P2 + P2 \cdot P3 - C_p) \cdot C_f - P2 \cdot C_p = 0 \quad (3)$$

The percentage free ibuprofen enantiomer in the original perfusate sample (f_u, %) was then calculated as

$$f_u(\%) = 100 \cdot C_f/C_p \quad (4)$$

Statistical Analysis

Differences in physiological function between the experimental groups were evaluated using a two-factor analysis of variance (ANOVA). Differences between the protein binding and clearance parameters of *R*(−)- and *S*(+)-ibuprofen were evaluated using a two-sample t test. A P value of ≤ 0.05 was considered significant.

RESULTS

The physiological function of the rat IPK was evaluated with respect to drug [*R*(−)- and *S*(+)-ibuprofen] and concentration (10 and 100 μg/ml). As shown in Table I, there were small but significant differences in perfusate flow due to drug and in $FE_{glucose}$ due to dose. Urine pH was statisti-

Table I. Effect of Ibuprofen (IB) Enantiomers on the Physiological Function of the Isolated Perfused Rat Kidney[a]

Parameter	Perfusate concentration: 10 μg/ml R(−)-IB	10 μg/ml S(+)-IB	100 μg/ml R(−)-IB	100 μg/ml S(+)-IB	Level of significance: Dose	Drug	Interaction
Perfusion pressure (mm Hg)	90.0 (4.9)	86.9 (3.1)	89.8 (3.6)	86.5 (1.5)	NS	NS	NS
Perfusate flow (ml/min)	37.0 (5.0)	46.7 (2.8)	39.7 (4.2)	45.6 (3.7)	NS	<0.01	NS
GFR (ml/min)	0.756 (0.060)	0.879 (0.109)	0.728 (0.063)	0.778 (0.129)	NS	NS	NS
$FE_{glucose}$ (%)	5.29 (1.00)	2.83 (0.68)	8.44 (4.44)	6.30 (3.43)	<0.05	NS	NS
Filtration fraction (%)	2.08 (0.17)	1.90 (0.26)	1.86 (0.39)	1.72 (0.38)	NS	NS	NS
Urine flow (ml/min)	0.116 (0.030)	0.112 (0.022)	0.137 (0.039)	0.111 (0.116)	NS	NS	NS
Urine pH	6.57 (0.07)	6.41 (0.07)	6.82 (0.06)	6.66 (0.06)	<0.01	<0.01	NS

[a] Data reported as the mean ± SD of four perfusion experiments. Each perfusion consists of eight to ten 10-min urine collection periods.

cally different with respect to dose and drug, but the magnitude of change was very small. Taken as a whole, the functionality of the preparation was remarkably stable. In particular, GFR was well maintained and consistent, indicating that there were no differences in functional nephron mass between the four treatment groups. $FE_{glucose}$ was not significantly different between the two enantiomers at both dose levels, indicating that tubular transport efficiency was unchanged as a function of drug. As also shown in Table I, parameters indicative of kidney function were within the normal range of values reported previously in control experiments with no drug present (6,16,17). Control experiments in the rat IPK ($n = 6$) performed at the time of drug studies had the following physiological function: perfusion pressure, 88.1 ± 2.8 mm Hg; perfusate flow, 40.0 ± 4.3 ml/min; GFR, 0.811 ± 0.160 ml/min; $FE_{glucose}$, 3.58 ± 1.42%; filtration fraction, 2.03 ± 0.35%; urine flow, 0.130 ± 0.025 ml/min; and urine pH, 6.41 ± 0.23.

The binding parameters ± SE of R(−)-ibuprofen were estimated as $P1 = 172 \pm 86$ μg/ml, $P2 = 2.06 \pm 1.32$ μg/ml, and $P3 = 37.8 \pm 6.6$; $r^2 = 0.996$. The binding parameters of S(+)-ibuprofen were estimated as $P1 = 229 \pm 125$ μg/ml, $P2 = 3.34 \pm 2.04$ μg/ml, and $P3 = 33.8 \pm 7.5$; $r^2 = 0.997$. The concentration-dependent protein binding that was observed for ibuprofen enantiomers was reflected by the 15–25% higher free fraction values at the 100-μg/ml as compared to the 10-μg/ml perfusate concentrations (Table II). The protein binding of ibuprofen was also stereoselective in that the percentage free was 10–20% greater for the S(+)-enantiomer.

Unchanged ibuprofen could not be detected in the urine unless the sample underwent an alkaline hydrolysis step. In addition, the oxidative metabolites of ibuprofen (hydroxy and carboxy) were sought but not found in selected perfusate and urine samples (detection limit of 0.10 μg/ml; 0.5-ml sample volume). As a result, the clearance of ibuprofen was equivalent to the apparent formation clearance of conjugated enantiomer. As shown in Table II, the clearance of R(−)-ibuprofen was substantially larger than its optical antipode (2.35 vs 0.805 μl/min, respectively; $P < 0.05$). The data were pooled since the clearance of R(−)-ibuprofen was not different at 10 and 100 μg/ml ($P > 0.50$). Furthermore, when the data were corrected for any potential differences in free fraction and GFR, the excretion ratio [$ER = CL_r/(f_u \cdot GFR)$] showed a similar finding. The R(−)-enantiomer had values for ER that were significantly greater than the S(+)-enantiomer (0.346 vs 0.0886, respectively; $P < 0.05$). ER data for R(−)-ibuprofen were also pooled since no concentration dependence was evident ($P > 0.50$).

Clearance data were not reported for S(+)-ibuprofen in the 10-μg/ml study because urine concentrations were below

Table II. Protein Binding and Clearance Parameters of Ibuprofen (IB) Enantiomers in the Isolated Perfused Rat Kidney[a]

Parameter	Perfusate concentration (μg/ml)	R(−)-IB	S(+)-IB	Significance
F_u (%)	10	0.844 (0.001)	0.990 (0.001)	$P < 0.001$
	100	1.05 (0.01)	1.15 (0.01)	$P < 0.001$
CL_r (μl/min)	10	2.50 (1.28)	—[c]	
	100	2.19 (1.42)	0.805 (0.290)	
	Pooled[b]	2.35 (1.26)		$P < 0.05$
ER	10	0.398 (0.209)	—[c]	
	100	0.295 (0.209)	0.0886 (0.0335)	
	Pooled[b]	0.346 (0.201)		$P < 0.05$

[a] Data reported as the mean ± SD of four perfusion experiments. Each perfusion consists of eight to ten 10-min urine collection periods.
[b] Data reported as the mean ± SD of eight perfusion experiments.
[c] Urine concentrations of ibuprofen were below the limit of assay sensitivity.

the limit of assay sensitivity. Still, using a detection limit of 0.10 μg/ml, the clearance of *S*(+)-enantiomer was estimated as ≤0.935 μl/min and ER as ≤0.107. These estimates represent upper limits and are similar to the calculated clearance and ER values for *S*(+)-ibuprofen in the 100-μg/ml study. Data on the disposition of *S*(+)-ibuprofen after treatment with *R*(−)-ibuprofen are absent because the chiral inversion process was lacking in the rat kidney.

DISCUSSION

Limited data are available concerning the stereoselective elimination of drugs by the renal route. In 1983, Lennard *et al.* (18) reported that the renal clearance of *R*-metoprolol was significantly higher (10%) than that of *S*-metoprolol in both extensive and poor debrisoquin metabolizers, although the reason for this difference was not discussed. A more substantial difference was subsequently demonstrated by Lima and co-workers (19) in which the unbound renal clearances of *S*(+)-disopyramide and *S*(+)-MND (mono-N-dealkylated metabolite) were approximately twice that of their *R*-enantiomers in man. Since the clearance values were higher than the filtration clearance expected in these subjects, it was concluded that disopyramide undergoes tubular secretion and that this process is stereoselective. In 1985, Hsyu and Giacomini (20) observed that the net secretion clearance of *l*-pindolol was, on average, 25% greater than that of *d*-pindolol in humans. Since the plasma protein binding was not different between enantiomers, they proposed that either stereoselective renal transport or stereoselective renal metabolism was occurring. Further support for stereoselectivity in renal tubular secretion was revealed by Notterman *et al.* (21) using diastereoisomeric cations. They observed in healthy volunteers that the unbound renal clearance of quinidine was 6.1 times that of creatinine and for quinine it was 1.5 times that of creatinine. However, caution should be used in interpreting the data for metoprolol (18), disopyramide (19), and quinidine/quinine (21) since the possibility of intrarenal metabolism was not considered.

In the present study, the excretory and metabolic profiles of ibuprofen enantiomers were explored in the rat IPK. Ibuprofen was chosen for study because of its documented stereochemical disposition and pharmacology, along with its potential for renal toxicity. As shown in Table II, the sum of renal mechanisms involved for the clearance of both *R*(−) and *S*(+)-enantiomers was net reabsorption. This is supported by values for excretion ratio of less than unity. However, it is possible that secretory transport also occurs since ibuprofen is recovered in the urine solely as conjugate, presumably as a glucuronide metabolite (1). Alternatively, filtered ibuprofen may be taken up and metabolized by the renal cells with subsequent excretion into the tubular fluid. Regardless, the renal elimination of ibuprofen appears to be stereoselective given the fact that its apparent formation clearance of conjugate and ER values are three to four times larger for the *R*(−)-enantiomer. Less clear is whether or not this stereoselectivity reflects differences in tubular transport of unchanged or conjugated ibuprofen, differences in metabolism once drug has been transported into the renal cell, or differences in cellular uptake.

As discussed above, prior studies (18–21) have reported on stereochemical differences in the renal transport of basic enantiomers and diastereomers. However, there is also evidence to support stereoselectivity of the glucuronidation process. For example, the formation of ester glucuronide conjugates is favored for *S*(+)-ibuprofen in human (22,23), *S*(+)-2-phenylpropionic acid in mice (24), and *S*-ketoprofen in humans (25,26). In contrast, the formation of the ester glucuronide is favored for *R*(−)-2-phenylpropionic acid in rat and rabbit (24), *R*-ketoprofen in rat (27), and *R*(−)-flurbiprofen in humans (28). As demonstrated in these examples and others (23), enantioselective differences with respect to glucuronidation may depend upon the NSAID in question and the animal model being studied.

A unique aspect of ibuprofen's disposition concerns its metabolic chiral inversion from the *R*(−)- to *S*(+)-enantiomer (2–4,22). Indeed, chiral inversion may occur with other NSAIDs, differing in rate and extent. As a result, ibuprofen has a greater *in vivo* S:R ratio as a function of time. This temporal flux can then impact on its potency since the drug's pharmacologic activity resides essentially with the *S*(+)-isomer. Given this scenario and ibuprofen's ability to cause adverse renal effects, we sought to determine if chiral inversion of ibuprofen enantiomers could occur in the kidney. No evidence for *R*- to *S*-inversion of ibuprofen was found in the rat IPK even though the kidney was suggested as an organ responsible for the optical isomerization of 2-phenylpropionic acid in the rat *in vivo* (29).

In conclusion, the results from this study demonstrate that the overall renal clearance mechanism of ibuprofen enantiomers was net reabsorption. Clearance data in the rat IPK agree with *in vivo* studies in the rat (30) in which the renal elimination of ibuprofen was negligible (<1% of total disposition). Ibuprofen was recovered in the urine solely as conjugated drug and there was no chiral inversion of *R*(−)- to *S*(+)-enantiomer. *R*(−)-Ibuprofen was cleared through the kidney faster than its optical antipode and may reflect differences in tubular transport of unchanged or conjugated drug species, differences in renal drug metabolism, or differences in cellular uptake.

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