Glucuronidation Kinetics of R,S-Ketoprofen in Adjuvant-Induced Arthritic Rats

Christine J. Meunier[1] and Roger K. Verbeeck[1,2]

Received December 14, 1998; accepted April 12, 1999

Purpose. A pharmacokinetic study was carried out in rats to investigate the effect of arthritis on the glucuronidation of the nonsteroidal anti-inflammatory drug ketoprofen.

Methods. An iv bolus dose of R,S-ketoprofen (10 mg/kg) was administered to control (n = 6) and adjuvant-induced arthritic rats (n = 6). All experiments were carried out in bile-exteriorized animals. Concentrations of R- and S-ketoprofen in plasma, bile and urine, and of their glucuronides in bile and urine were determined by HPLC. In a separate series of experiments, the ex vivo plasma protein binding of R- and S-ketoprofen was measured in control and arthritic rats following iv administration of R,S-ketoprofen.

Results. As a result of a significant decrease in plasma albumin concentrations in arthritic rats, the unbound fraction of R- and S-ketoprofen was significantly increased (approximately 2-fold) in rats with adjuvant-induced arthritis. Total (i.e., bound plus unbound) plasma clearances of R- and S-ketoprofen were not different in arthritic rats. Unbound plasma clearances of both ketoprofen enantiomers, however, were significantly reduced (by 53% and 61%, respectively). This was due to a significant impairment in the formation of the R- and S-ketoprofen glucuronides. There was no apparent effect of adjuvant-induced arthritis on the chiral inversion of R- to S-ketoprofen.

Conclusions. Adjuvant-induced arthritis in the rat leads to a significant impairment in the in vivo glucuronidation of R- and S-ketoprofen.

KEY WORDS: adjuvant-induced arthritis; R,S-ketoprofen; glucuronidation.

INTRODUCTION

Adjuvant-induced arthritis in the rat is an accepted model for rheumatoid arthritis (1,2). Several studies have shown that the pharmacokinetics of a number of drugs are altered in rats with adjuvant-induced arthritis (e.g., 3–6). Mechanisms involved in these altered pharmacokinetics include changes in plasma protein binding and impaired metabolism by cytochrome P450. No information is available in the literature concerning the effect of adjuvant-induced arthritis on *in vivo* drug glucuronidation, a phase II metabolic pathway which is important for the elimination of many xenobiotics and/or their phase I metabolites. However, Toda *et al.* (7) have demonstrated a 50% reduction in the *in vitro* hepatic microsomal glucuronidation of p-nitrophenol in adjuvant arthritic rats whereas the glucuronidation of bilirubin was not affected. We have recently shown that adjuvant-induced arthritis did not influence the hepatic microsomal glucuronidation of acetaminophen (8). The hepatic microsomal glucuronidation of p-nitrophenol, ketoprofen and diflunisal, on the contrary, was reduced by approximately 50% in adjuvant-induced arthritic rats (8). These observations demonstrate that the effect of adjuvant-induced arthritis on glucuronidation seems to be substrate dependent. In addition, β-glucuronidase catalyzed hydrolysis of glucuronides has been demonstrated to affect the *in vivo* pharmacokinetics of substances in the rat (9). Since earlier studies have shown an increased β-glucuronidase activity in plasma of patients with rheumatoid arthritis (10), it would be worthwhile to measure activities of this hydrolase in plasma, urine and bile in an attempt to explain possible alterations in the pharmacokinetic behavior of R- and S-ketoprofen and their glucuronides in adjuvant-induced arthritis.

The objective of the present study, therefore, was to investigate the possible consequences of the in vitro observation that ketoprofen glucuronidation is significantly impaired in adjuvant-induced arthritis for the *in vivo* pharmacokinetic characteristics of this nonsteroidal anti-inflammatory drug. Since enterohepatic cycling of R,S-ketoprofen via biliary excretion of their glucuronides has been demonstrated in the rat (11), the pharmacokinetic experiments were carried out in bile-exteriorized animals.

MATERIALS AND METHODS

Chemicals

R,S-ketoprofen and α-naphthyl-β-D-glucuronide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). R,S-ketoprofen glucuronide (R,S-KG) was isolated and purified from urine of a volunteer after ingestion of a 100 mg commercial ketoprofen capsule (Rofenid®, Rhône Poulenc Rorer, France) and used to prepare standards for calibration curves. The identity of the isolated material was confirmed by mass spectrometry (fast atom bombardment) and by alkaline hydrolysis to R- and S-K. HPLC grade isopropyl alcohol and diethyl ether were purchased from Labscan (Dublin, Ireland). All other chemicals were of the highest purity available from standard commercial sources.

Animal Experiments

Male Wistar rats (Charles River France, St. Aubin-lès-Elbeuf, France), weighing between 160 and 180 grams, received an injection of Mycobacterium butyricum suspended in liquid paraffin (0.5 mg/0.1 ml) into the tail base. Control animals were injected with an equivalent volume of vehicle. The rats (6 animals in each treatment group) were maintained at 21 ± 2°C on a 12 hour light-dark cycle and had free access to food (type AO3, UAR, Epinay-sur-Orge, France) and water until arthritis, assessed by measuring the circumference of the right and left ankles, had developed (between 20 to 28 days after injection of adjuvant) (8). All experimental procedures were approved by the University Animal Experimentation Ethics Committee.

Under pentobarbital anesthesia (50 mg/kg ip) Silclear® catheters (0.020″ × 0.037″) were placed in the two external jugular veins, one for iv drug administration and the other for blood sampling. In addition, a PE-50 cannula (0.023″ × 0.038″)

[1] Pharmacokinetics and Drug Metabolism Laboratory, School of Pharmacy, Université Catholique de Louvain, (UCL), Brussels, Belgium.
[2] To whom correspondence should be addressed. (e-mail: verbeeck@pmnt.ucl.ac.be)

0724-8741/99/0700-1081$16.00/0 © 1999 Plenum Publishing Corporation

was inserted into the bile duct for bile collection (12). The animals were allowed to recover from surgery overnight. Each rat received an iv bolus injection of R,S-ketoprofen (10 mg/kg) dissolved in polyethyleneglycol 400/isotonic saline (40/60). Blood samples were withdrawn in syringes containing heparin at the following times: 0 (pre-dose), 2.5, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes following drug administration. Blood samples were immediately centrifuged and the plasma was frozen at −20°C until analysis. Total urine and total bile output were collected on ice into preweighed vials containing 0.5–1.0 ml of 1 M acetic acid. Bile and urine samples were immediately frozen at −20°C until analysis.

Ex Vivo Determination of the Plasma Protein Binding of R- and S-Ketoprofen

To determine the unbound fraction of the ketoprofen enantiomers in plasma, an additional 16 rats, having the same specifications and having undergone the same surgical preparations as described above, received an iv bolus of 10 mg/kg R,S-ketoprofen. A single 6 ml blood sample (i.e., each rat was only sampled once) was withdrawn from 2 control and 2 arthritic rats in a syringe containing heparin at the following times after drug administration: 0 (blank), 5, 30 and 90 minutes. These blood samples were immediately centrifuged and the plasma stored at −20°C. Plasma protein binding was determined by equilibrium dialysis (4 hours) at 37°C in a Dianorm Equilibrium Dialyzer equipped with 1 ml teflon cells separated by a Spectra/Por 2 semi-permeable membrane (Spectrum Medical Industries, Houston, TX, USA). Albumin plasma concentrations were determined before and after dialysis by the bromocresol green binding method (13) and used to correct the unbound fraction for volume shifts (14).

Analytical Procedures

Concentrations of R- and S-ketoprofen in plasma, urine, bile and dialysate were determined according to a method described by Menzel-Soglowek (15). Briefly, a known volume of plasma (50 μl), urine (100 μl), bile (100 μl) or dialysate (400 μl) was acidified by adding 200 μl of 2 M HCl containing the internal standard α-naphthyl-β-D-glucuronide (250 μg/ml) and extracted with 6 ml of ice-cooled diethyl ether. After centrifugation (1500 × g, 10 min, 4°C), 5 ml of the organic layer were transferred to a clean tube and evaporated to dryness under a gentle stream of nitrogen at room temperature. The residue was dissolved in 250 to 500 μl of mobile phase and 50 μl injected onto a CHIRAL-AGP column (5 μm, 100 × 4 mm, ChromTech AB, Hägersten, Sweden) maintained at 15°C (plasma, bile, dialysate) or 13°C (urine). The mobile phase was composed of 0.5% isopropyl alcohol in 20 mM NaH_2PO_4 buffer containing 0.1 M NaCl and 5 mM N,N-dimethyloctylamine and adjusted to pH 6.7 (plasma, bile, dialysate) or pH 6.0 (urine). The flow rate was 0.7 ml/min (plasma, bile, dialysate) or 0.9 ml/min (urine), and the eluent was monitored at 260 nm. Standards (5, 10, 25, 50, 75 and 100 μg/ml) prepared in blank plasma, urine, bile and dialysis buffer were always analyzed together with the "experimental" biological samples. Inter- and intra-day coefficients of variation for the quantification of R- and S-ketoprofen in plasma, urine, bile and dialysate, measured at 5, 50 and 100 μg/ml, were always smaller than 10%. Concentrations of ketoprofen glucuronides were determined after alkaline hydrolysis (16).

Albumin concentrations were determined in the pre-dose (i.e., 0 time) plasma samples of both control and arthritic rats by the bromocresol green binding method (13). β-Glucuronidase activity was measured in pre-dose plasma, bile and urine samples of control and arthritic rats using 4-methylumbelliferone glucuronide as substrate (17).

Data Analysis

Pharmacokinetic parameters were calculated by the so-called "noncompartmental" approach. AUC values were estimated using the linear trapezoidal rule from 0 to t (time of last blood sampling) with extrapolation to infinity (plasma concentration at time t divided by λ_z). The terminal plasma half-life ($t1/2_z$) was calculated as $0.693/\lambda_z$ and λ_z is estimated by linear regression of the terminal log-linear phase of the plasma concentration-time curve. Plasma clearance (CL) was calculated as dose/AUC and apparent volume of distribution (Vd) as CL/λ_z. The fraction of the iv administered dose excreted in urine and bile as unchanged R- and S-ketoprofen or their glucuronides ($f_{r.X}$ and $f_{b.X}$, respectively) was calculated as the total amount of substance X (unchanged drug or glucuronide conjugate expressed as drug equivalents) recovered in urine or bile divided by the iv administered dose. Renal and biliary clearances (Cl_r and Cl_b, respectively) were calculated as the amount of R-ketoprofen (or S-ketoprofen) recovered in urine or bile divided by the AUC of R-ketoprofen (or S-ketoprofen). Since the plasma concentrations of the glucuronides of R- and S-ketoprofen were too low to quantify, the renal and biliary clearances of the glucuronides could not be calculated. The formation clearances (CL_m) of R- and S-ketoprofen glucuronide were calculated as $CL.f_{m.R\text{-}KG}$ and $CL.f_{m.S\text{-}KG}$, respectively, where $f_{m.R\text{-}KG}$ (or $f_{m.S\text{-}KG}$) represents the fraction of the iv drug dose converted to R-ketoprofen glucuronide (or S-ketoprofen glucuronide), i.e., the combined urinary plus biliary recoveries. The unbound plasma clearance of R- and S-ketoprofen (Clu) was obtained as CL/fu, where fu is the fraction unbound in plasma.

Values in the text, tables and figures are expressed as mean ± SEM. Comparison of mean parameters between control and arthritic rats was performed by unpaired t-test. Linear regression analysis was carried out to look for a possible correlation between the fraction of drug unbound in plasma and the plasma albumin concentration. A p-value of 0.05 or less was considered significant.

RESULTS

Ketoprofen pharmacokinetics were stereoselective following iv bolus injection of R,S-ketoprofen (10 mg/kg): R-ketoprofen showed a much higher plasma clearance (7.5 ± 1.1 ml/min·kg) and higher distribution volume (206.4 ± 21.1 ml/kg) compared to S-ketoprofen (2.5 ± 0.3 ml/min·kg and 119.4 ± 4.4 ml/kg, respectively) (Table I). Whereas plasma concentration-time profiles of R-ketoprofen were very similar in control and arthritic rats, S-ketoprofen plasma concentrations were somewhat higher in the arthritic rats (Fig. 1A). For both R- and S-ketoprofen, the pharmacokinetic parameters CL, Vd and λ_z, calculated based on total (i.e. bound plus unbound) plasma

Table I. Pharmacokinetic Parameters of Total (i.e., Bound Plus Unbound) R- and S-Ketoprofen Following iv Bolus Injection of R,S-Ketoprofen (10 mg/kg) in Control (n = 6) and Arthritic (n = 6) Rats

Parameter	R-Ketoprofen		S-Ketoprofen	
	Control	Arthritis	Control	Arthritis
$AUC_{0-\infty}$ (μg·h/ml)	12.6 ± 2.0	13.8 ± 3.1	36.4 ± 4.7	48.3 ± 7.3
CL (ml/min·kg)	7.50 ± 1.13	7.01 ± 0.91	2.49 ± 0.33	1.94 ± 0.30
Vd (ml/kg)	206.4 ± 21.1	246.6 ± 11.8	119.4 ± 4.4	138.6 ± 9.6
λ_z (1/h)	2.22 ± 0.34	1.69 ± 0.20	1.26 ± 0.18	0.86 ± 0.15
Cl_r (ml/min·kg)	0.024 ± 0.003	0.055 ± 0.010*	0.006 ± 0.001	0.019 ± 0.006
Cl_b (ml/min·kg)	0.10 ± 0.02	0.12 ± 0.02	0.11 ± 0.01	0.07 ± 0.01
CL_m[a]	0.96 ± 0.14	0.63 ± 0.09	2.07 ± 0.29	1.43 ± 0.26

[a] partial clearance for the formation of R-KG and S-KG.
* $p < 0.05$.

concentrations were, however, not statistically significantly affected by adjuvant-induced arthritis (Table I).

Plasma albumin concentrations were significantly ($p < 0.005$) lower in arthritic rats (2.29 ± 0.07 g/100 ml and 2.32 ± 0.07 g/100 ml for the *ex vivo* plasma binding study and the pharmacokinetic study, respectively) compared to control rats (3.11 ± 0.08 g/100 ml and 3.08 ± 0.09 g/100 ml, respectively). As a result, the unbound fractions of R- and S-ketoprofen in plasma were significantly increased in arthritic rats (0.113 ± 0.005 and 0.118 ± 0.004 for R- and S-ketoprofen, respectively) compared to control rats (0.047 ± 0.004 and 0.054 ± 0.004, respectively). The unbound fractions of R- and S-ketoprofen, determined *ex vivo*, were relatively constant as a function of time following iv bolus injection of R,S-ketoprofen (10 mg/kg), indicating that the plasma binding of the ketoprofen enantiomers was not concentration dependent (Table II) under the conditions of

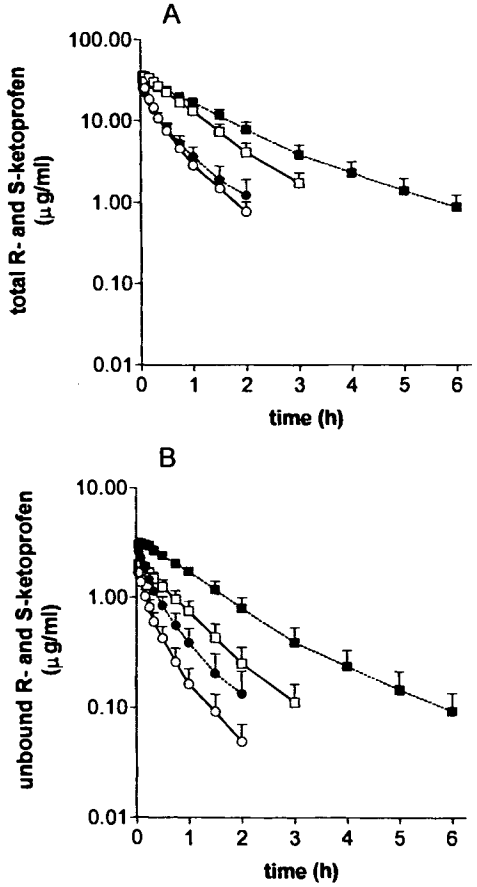

Fig. 1. Mean semi-logarithmic total (A) and unbound (B) plasma concentration-time profiles of R-ketoprofen (○, ●) and S-ketoprofen (□, ■) following iv bolus injection of R,S-ketoprofen (10 mg/kg) to bile-exteriorized control (○, □) (n = 6) and arthritic rats (●, ■)(n = 6).

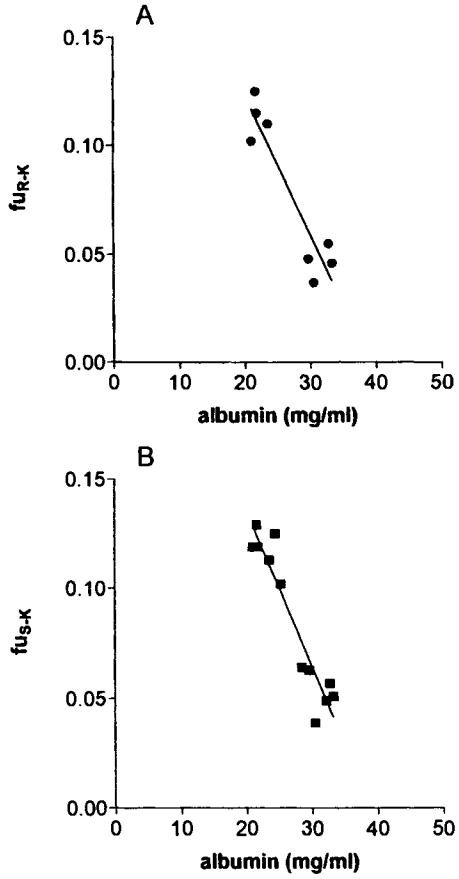

Fig. 2. Linear correlation analysis between unbound plasma fractions of R-ketoprofen, fu_{R-K}, (graph A) and S-ketoprofen, fu_{S-K} (graph B) in control and arthritic rats and plasma albumin concentrations (A: r = 0.93, $p < 0.005$, n = 8; B: r = 0.94, $p < 0.005$, n = 12).

Table II. Unbound Fractions of R-and S-Ketoprofen Determined by Equilibrium Dialysis in the Plasma of Control Rats and Arthritic Rats 5 min, 30 min and 90 min After iv Bolus Administration of R,S-Ketoprofen (10 mg/kg)

Time	$fu_{R\text{-}K}$		$fu_{S\text{-}K}$	
	Control	Arthritis	Control	Arthritis
5 min	0.047	0.125	0.051	0.129
	0.048	0.102	0.063	0.119
30 min	0.055	0.115	0.057	0.119
	0.037	0.110	0.039	0.113
90 min	—[a]	—	0.064	0.125
	—	—	0.049	0.102
mean ± SEM	0.047 ± 0.004	0.113 ± 0.005*	0.054 ± 0.004	0.118 ± 0.004*

Note: At each time point 2 control rats and 2 arthritic rats were sampled.
[a] Dialysate concentrations of R-ketoprofen below the limit of quantification.
* $p < 0.005$.

the in vivo pharmacokinetic study. Linear regression analysis revealed a simple linear relationship between the plasma albumin concentration and the unbound fraction of R-ketoprofen ($r = 0.93$, $p < 0.005$, $n = 8$) and S-ketoprofen ($r = 0.94$, $p < 0.005$, $n = 12$) in the individual control and arthritic rats (Fig. 2). The parameters of the regression line were subsequently used to estimate the unbound fractions of R- and S-ketoprofen in the control and arthritic rats undergoing the pharmacokinetic experiment as a function of their individual plasma albumin concentrations. Based on these estimated fu values, total plasma concentrations of the ketoprofen enantiomers as determined in the pharmacokinetic experiments were converted to unbound plasma concentrations (Fig. 1B) and the unbound plasma clearance was calculated. Adjuvant-induced arthritis resulted in a significant reduction in the unbound plasma clearance of both R- and S-ketoprofen (Table III).

Urinary and biliary recoveries of R- and S-ketoprofen and their glucuronides are summarized in Table IV. Only small quantities of unchanged R- and S-ketoprofen were excreted via urine (<1–2% of the administered dose) or bile (<6%) in control and arthritic rats. Most of the iv administered R,S-ketoprofen dose was recovered in the bile of control rats as the glucuronide of S-ketoprofen (82.2 ± 1.1%). Biliary excretion of the R-ketoprofen glucuronide accounted for 12.6 ± 0.6% of the administered dose. Urinary excretion of the ketoprofen glucuronides was negligible (<0.5% of the administered dose) in control rats. Adjuvant-induced arthritis resulted in a statistically significant reduction of the biliary excretion of R- and S-ketoprofen glucuronide (−40% and −21%, respectively). The urinary excretion of R- and S-ketoprofen glucuronide was statistically significantly increased in arthritic rats but remained relatively small (Table IV).

Based on these urinary and biliary recoveries, the renal and biliary clearances of R- and S-ketoprofen ware calculated as well as the partial metabolic clearances for formation of R- and S-ketoprofen glucuronides. These clearances were calculated based on total (Table I) and unbound (Table III) plasma concentrations of the ketoprofen enantiomers. Significant differences, especially in some of the unbound clearances, could be demonstrated between control and arthritic rats. For example, the unbound biliary clearances of R- and S-ketoprofen were sigificantly impaired in arthritic rats (1.18 ± 0.23 ml/min·kg and 0.63 ± 0.12 ml/min·kg, respectively) compared to control rats (1.96 ± 0.27 ml/min·kg and 1.83 ± 0.27 ml/min.·g, respectively). In adjuvant-induced arthritis the unbound partial metabolic clearances for the formation of both R- and S-ketoprofen glucuronides were decreased to one third of the control value (Table III).

Finally, adjuvant-induced arthritis did not result in statistically significant changes in β-glucuronidase activities of plasma, urine or bile (Table V). However, although statistical

Table III. Pharmacokinetic Parameters of Unbound R- and S-Ketoprofen Following iv Bolus Injection of R,S-Ketoprofen (10 mg/kg) in Control (n = 6) and Arthritic (n = 6) Rats

Parameter	R-Ketoprofen		S-Ketoprofen	
	Control	Arthritis	Control	Athritis
fu[a]	0.054 ± 0.006	0.102 ± 0.005***	0.060 ± 0.006	0.113 ± 0.005***
Clu (ml/min.kg)	146 ± 24	69 ± 9*	44 ± 7	17 ± 2***
Clu_r (ml/min.kg)	0.48 ± 0.09	0.54 ± 0.11	0.11 ± 0.02	0.16 ± 0.05
Clu_h (ml/min.kg)	1.96 ± 0.27	1.18 ± 0.23*	1.83 ± 0.27	0.63 ± 0.12***
CLu_m[b]	18.8 ± 2.9	6.1 ± 0.8**	36.6 ± 6.1	12.4 ± 1.9**

[a] Estimated based on the rat's plasma albumin concentration and the correlation demonstrated between fu and plasma albumin concentration in the ex vivo binding experiments (see also Fig. 2).
[b] Partial clearance for the formation of R-KG and S-KG.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.005$.

significance was not reached, it should be pointed out that control rats had substantially lower urinary β-glucuronidase activities (5.7 ± 1.2 units/ml, range: 0.4–9.1 units/ml) compared to arthritic rats (16.1 ± 6.5 units/ml, range: 1.5–42.3 units/ml).

DISCUSSION

Ketoprofen, a 2-arylpropionic acid derivative, is highly bound to albumin and mainly eliminated by glucuronidation. It is administered as a racemic mixture and undergoes substantial unidirectional metabolic inversion of the R-enantiomer to the S-enantiomer in the rat (18). In addition, the R- and S-ketoprofen glucuronides are mainly excreted via the bile and R- and S-ketoprofen are reabsorbed following hydrolysis of the ketoprofen glucuronides in the gut (enterohepatic circulation) (11). The biliary excretion of R- and S-ketoprofen glucuronides, their subsequent hydrolysis and the reabsorption of the unchanged ketoprofen enantiomers were shown to be highly stereoselective (19). It was decided to study the disposition kinetics of R- and S-ketoprofen in bile-duct cannulated rats because: (1) it would then be possible to directly measure the biliary excretion of the ketoprofen glucuronides, which is their main excretion route, and (2) it would greatly simplify the pharmacokinetic interpretation of the data. The results of our studies in control rats are consistent with previous reports regarding the stereoselectivity in the pharmacokinetics of ketoprofen (11,18,19). The fact that approximately 82% of the iv administered R,S-ketoprofen dose was recovered in bile as the S-ketoprofen glucuronide is proof of the important chiral inversion of R-to S-ketoprofen in the rat.

Adjuvant-induced arthritis did not result in a significant change in the plasma clearance of total (i.e., bound plus unbound) R- and S-ketoprofen. Since R-and S-ketoprofen are compounds with a low hepatic extraction ratio (their plasma clearances are low compared to hepatic plasma flow), their total plasma clearances (CL) are the product of the unbound plasma fraction (fu) and the unbound plasma clearance (CLu). CLu is therefore a better parameter to characterize the intrinsic capacity of the liver to eliminate (i.e. glucuronidate) R- and S-ketoprofen. To correctly estimate CLu of R- and S-ketoprofen, the plasma protein binding of both enantiomers should be measured. Therefore, a second parallel study was carried out allowing the determination of the effect of adjuvant-induced arthritis on the *ex vivo* plasma protein binding of the ketoprofen enantiomers. Induction of adjuvant-induced arthritis resulted in significantly decreased plasma albumin concentrations leading to a more than doubling of the unbound plasma fractions of the ketoprofen enantiomers. Consequently, the unbound plasma clearances of R- and S-ketoprofen were reduced by 53% and 61%, respectively. The unbound partial metabolic clearances for the formation of the glucuronide conjugates were also reduced to a similar extent: 68% and 66% for the formation of the R- and S-ketoprofen glucuronide, respectively.

β-Glucuronidase activities have been reported to be increased in tissues of adjuvant-induced arthritic rats (20). Results of the present study, however, did not reveal any significant changes in the β-glucuronidase activities in plasma or bile of arthritic rats. Urinary β-glucuronidase activities were on average almost 3 times higher in arthritic rats. This observation may explain the significant increases (2- to 3-fold) in urinary recoveries of unchanged R- and S-ketoprofen in arthritic rats. However, the urinary recovery of unchanged R- and S-ketoprofen was negligible, even in arthritic rats (0.8% and 0.9%, respectively), and is probably the result of spontaneous or increased β-glucuronidase catalyzed hydrolysis of the ketoprofen glucuronides excreted in urine (21,22). Biliary excretion of unchanged R-and S-ketoprofen was also relatively unimportant (approximately 1.5% and 4%, respectively) and was not affected by adjuvant-induced arthritis. Again, it is not clear whether recovery of unchanged R- and S-ketoprofen is due to direct biliary excretion of these compounds or the result of spontaneous or β-glucuronidase catalyzed hydrolysis of the R- and S-ketoprofen glucuronides following their excretion into bile.

Table V. β-Glucuronidase Activities (units[a]/ml) in Plasma, Urine and Bile of Control (n = 6) and Arthritic Rats (n = 6)

	Control rats	Arthritic rats
Plasma	1.69 ± 0.20	1.67 ± 0.26
Urine	5.7 ± 1.2	16.1 ± 6.5
Bile	0.03 ± 0.01	0.02 ± 0.01

[a] 1 unit corresponds to 1 μg 4-methylumbelliferone liberated at 37°C from 4-methylumbelliferyl glucuronide per hour and per ml of plasma, urine or bile.

Table IV. Urinary (f_r) and Biliary Recoveries (f_b) of R-Ketoprofen (R-K), S-Ketoprofen (S-K), R-Ketoprofen Glucuronide (R-KG) and S-Ketoprofen Glucuronide (S-KG) Following iv Bolus Administration of R,S-Ketoprofen (10 mg/kg) in Control (n = 6) and Arthritic Rats (n = 6)

	f_r		f_b		f_{r+b}	
	Control	Arthritis	Control	Arthritis	Control	Arthritis
R-K	0.30 ± 0.04	0.80 ± 0.09***	1.5 ± 0.2	1.7 ± 0.2	1.8 ± 0.2	2.4 ± 0.2
S-K	0.30 ± 0.05	0.90 ± 0.30*	4.3 ± 0.4	3.6 ± 0.5	4.6 ± 0.4	4.6 ± 0.7
R-KG	0.40 ± 0.05	1.6 ± 0.30**	12.6 ± 0.6	7.5 ± 0.8***	13.0 ± 0.6	9.2 ± 0.9**
S-KG	0.20 ± 0.08	7.1 ± 2.3*	82.2 ± 1.1	65.3 ± 5.0*	82.4 ± 1.2	72.3 ± 4.4*
total	1.20 ± 0.08	10.4 ± 2.7*	100.6 ± 0.7	78.1 ± 5.4**	101.8 ± 0.8	88.5 ± 4.3*

Note: Recoveries are tabulated as percent of total (i.e., R-ketoprofen plus S-ketoprofen) administered dose.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.005$.

Biliary recovery of the ketoprofen glucuronides was significantly reduced in adjuvant-induced arthritis. Since plasma concentrations of R- and S-ketoprofen glucuronide were below the limit of quantification, biliary (and renal) clearances of the ketoprofen glucuronides could not be calculated. However, it seems likely that adjuvant-arthritis resulted in impaired biliary transport of the ketoprofen glucuronides. Urinary recovery of R- and S-ketoprofen glucuronides, on the contrary, was significantly increased in arthritic rats (4-fold and 35-fold, respectively). This could theoretically be the result of decreased plasma protein binding of the ketoprofen glucuronides and/or a compensatory increase in urinary excretion due to impaired biliary excretion.

In conclusion, the glucuronidation of both R- and S-ketoprofen is significantly impaired in adjuvant-arthritic rats. These results confirm our recent *in vitro* finding that the glucuronidation of both ketoprofen enantiomers by rat liver microsomes is significantly impaired in adjuvant-induced arthritis (8). In addition, our observation that unbound concentrations of R- and S-ketoprofen in plasma are higher in arthritic rats is consistent with results obtained with naproxen, a 2-arylpropionic acid derivative mainly eliminated in man by glucuronidation, in patients with rheumatoid arthritis (23,24). It would therefore be interesting to study the pharmacokinetics of total and unbound R- and S-ketoprofen in patients with rheumatoid arthritis to see whether the adjuvant-induced arthritic rat is a good model to predict drug glucuronidation in rheumatoid arthritis.

ACKNOWLEDGMENTS

The authors thank Mrs. M. Petit for her excellent technical assistance.

REFERENCES

1. M. W. Whitehouse. Adjuvant-induced polyarthritis in rats. In "*Handbook of Animal Models for the Rheumatic Diseases*", vol I (Eds. R. Greenwald and H. Diamond), CRC Press, Boca Raton, pp. 3–16, 1988.
2. B. M. Weichman. Rat adjuvant arthritis: a model of chronic inflammation. In "*Pharmacological Methods in the Control of Inflammation*" (Eds. J. Y. Chang and A. J. Lewis), Alan R. Liss, Inc., New York, pp. 363–380, 1989.
3. K. A. Walker, H. E. Barber, and G. M. Hawksworth. Mechanism responsible for altered propranolol disposition in adjuvant-induced arthritis in the rat. *Drug Metab. Disp.* **14**:482–486 (1986).
4. S. H. Pollock, H. W. Matthews, and M. J. D'Souza. Pharmacokinetic analysis of cyclosporine in adjuvant arthritic rats. *Drug Metab. Disp.* **17**:595–599 (1989).
5. M. Piquette-Miller and F. Jamali. Effect of adjuvant arthritis on the disposition of acebutolol enantiomers in rats. *Agents Actions* **37**:290–296 (1992).
6. M. Piquette-Miller and F. Jamali. Influence of severity of inflammation on the disposition kinetics of propranolol enantiomers in ketoprofen-treated and untreated adjuvant arthritis. *Drug Metab. Disp.* **23**:240–245 (1995).
7. A. Toda, N. Ishii, T. Kihara, A. Nagamatsu, and H. Shimeno. Effect of adjuvant-induced arthritis on hepatic drug metabolism in rats. *Xenobiotica* **26**:603–611 (1994).
8. C. J. Meunier and R. K. Verbeeck. Glucuronidation of R- and S-ketoprofen, acetaminophen and diflunisal by liver microsomes of adjuvant-induced arthritic rats. *Drug Metab. Disp.* **27**:26–31 (1999).
9. F. M. Brunelle and R. K. Verbeeck. Conjugation-deconjugation cycling of diflunisal via β-glucuronidase catalyzed hydrolysis of its acyl glucuronide in the rat. *Life Sci.* **60**:2013–2021 (1997).
10. A. Falkenbach, U. Unkelbach, and R. Gottschalk. β-Glucuronidase-Konzentration im Serum als potentieller Indikator der Krankheitsaktivität bei rheumatoider Arthritis (RA) und systemischem Lupus erythematodes (sLE). *Med. Klin.* **86**:465–468 (1991).
11. H. Yasui, K. Yamaoka, and T. Nakagawa. Moment analysis of stereoselective enterohepatic circulation and unidirectional chiral inversion of ketoprofen enantiomers in rat. *J. Pharm. Sci.* **85**:580–585 (1996).
12. S. L. Eeckhoudt, P. A. Evrard, and R. K. Verbeeck. Biliary excretion and enterohepatic cycling of R- and S-flurbiprofen in the rat. *Drug Metab. Disp.* **25**:428–430 (1997).
13. R. Corcoran and S. Duran. Albumin determination by a modified bromocresol green method. *Clin. Chem.* **23**:765–766 (1977).
14. K. M. Giacomini, F. M. Wong, and T. N. Tozer. Correction for volume shift during equilibrium dialysis by measurement of protein concentration. *Pharm. Res.* **4**:179–181 (1984).
15. S. Menzel-Soglowek, G. Geisslinger, and K. Brune. Stereoselective high-performance liquid chromatographic determination of ketoprofen, ibuprofen and fenoprofen in plasma using a chiral α_1-glycoprotein column. *J. Chromatogr.* **532**:295–303 (1990).
16. R. T. Foster and F. Jamali. High performance liquid chromatographic assay of ketoprofen enantiomers in human plasma and urine. *J. Chromatogr.* **416**:388–393 (1987).
17. J. A. R. Mead, J. N. Smith, and R. T. Williams. The biosynthesis of the glucuronides of umbelliferone and 4-methylumbelliferone and their use in the fluorimetric determination of β-glucuronidase. *Biochem. J.* **61**:569–574 (1955).
18. R. T. Foster and F. Jamali. Stereoselective pharmacokinetics of ketoprofen in the rat. Influence of route of administration. *Drug Metab. Disp.* **16**:623–626 (1988).
19. S. Menzel, W. S. Beck, K. Brune, and G. Geisslinger. Stereoselectivity of biliary excretion of 2-arylpropionates in rats. *Chirality* **5**:422–427 (1993).
20. G. K. Reddy and S. C. Dhar. Metabolism of glycosaminoglycans in tissues of adjuvant arthritic rats. *Mol. Cell. Biochem.* **106**:117–124 (1991).
21. R. A. Upton, J. N. Buskin, R. L. Williams, N. H. G. Holford, and S. Riegelman. Negligible excretion of unchanged ketoprofen, naproxen, and probenecid in urine. *J. Pharm. Sci.* **11**:1254–1257 (1980).
22. R. G. Dickinson and A. R. King. Vesico-hepato-renal cycling of acidic drugs via their reactive acyl glucuronide metabolites? Studies with diflunisal in rats. *Clin. Exp. Pharmacol. Physiol.* **23**:665–668 (1996).
23. F. A. Van Den Ouweland, M. J. A. Franssen, L. B. A. Van De Putte, Y. Tan, C. A. M. Van Ginneken, and F. W. J. Gribnau. Naproxen pharmacokinetics in patients with rheumatoid arthritis during active polyarticular inflammation. *Br. J. Clin. Pharmacol.* **23**:189–193 (1987).
24. F. A. Van Den Ouweland, F. W. J. Gribnau, C. A. M. Van Ginneken, Y. Tan, and L. B. A. Van De Putte. Naproxen kinetics and disease activity in rheumatoid arthritis: a within-patient study. *Clin. Pharmacol. Ther.* **43**:79–85 (1988).