Pharmacokinetic Analysis of Enterohepatic Circulation of Etdolac and Effect of Hepatic and Renal Injury on the Pharmacokinetics

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Received October 2, 1996; accepted December 20, 1996.

This study was designed to evaluate the enterohepatic circulation of racemic etdolac in rats. Additionally, the effect of hepatic and renal failure on the pharmacokinetics was estimated. The biliary excretion and the reabsorption of the drug excreted in bile were examined in order to clarify the effect of enterohepatic circulation on the disposition, and a pharmacokinetics model was applied to describe the enterohepatic circulation. The relatively rapid elimination of etdolac was seen in the bile-exuoriated rats (BE rat) compared with that in control rats. Total biliary excretion of etdolac, mainly as glucuronides, after intravenous administration was about 45% of the dose, indicating extensive enterohepatic circulation of the drug. The plasma concentrations of the drug in bile duct-linked rats approximately agreed with the simulation curve by the model, with the peak concentration 6–7 h after dosing. The elimination of the drug was markedly retarded in rats with hepatic (CCL4-induced) and renal (uranyl acetate-induced) failure, and high plasma levels were maintained over the longer times, due to greatly decreased distribution volume. The biliary excretion of etdolac enantiomers was not significantly different between the control and CCl4-groups, suggesting that hepatic glucuronyl transferase activity was preserved in rats impaired by CCl4.

Key words etdolac; hepatic failure; renal failure; enterohepatic circulation model; enantiomer biliary excretion

Etdolac is an effective and well-tolerated nonsteroidal anti-inflammatory drug (NSAID) which is marketed commercially as the racemic mixture of R- and S-enantiomers. Etdolac has previously been shown to exhibit a high degree of stereoselectivity in its pharmacokinetics in human1–3 and in rats.4,5 The pharmacological activities of NSAIDs are mainly, if not entirely, associated with the S-enantiomer.6–8

Pharmacologically active S-etdolac has a significant smaller area under the plasma concentration (AUC), greater distribution volume (Vd) and longer half-life (t1/2) than does the inactive R-enantiomer after intravenous (i.v.) administration of racemic etdolac to the rat.9 This stereoselectivity has been ascribed to differences between enantiomers in metabolism, plasma protein binding, and biliary excretion. Although almost all of the administered S-enantiomer was recovered in rat bile as glucuronide metabolite, only 30% of the R-enantiomer was recovered after dosing of racemate, and R-enantiomer is suggested to be subject to metabolism to oxidized metabolites.9 However, the enterohepatic circulation of etdolac has not been demonstrated, although it is suggested that S-enantiomer undergoes enterohepatic circulation.4,10 Additionally, the effect of hepatic and renal disease on the pharmacokinetics of etdolac has not been well-characterized.

In this study we attempted to clarify the enterohepatic circulation of racemic etdolac in rats. The pharmacokinetics of the drug was also estimated in rat with hepatic and renal failure to evaluate the effect of these conditions on the disposition. The biliary excretion and the plasma elimination of etdolac in bile-exuoriated rats, and reabsorption of the drug excreted in bile were examined to determine the effect of enterohepatic circulation on the plasma kinetics, and a pharmacokinetic model was applied to describe the reabsorption of the drug excreted in bile. In some experiments, the stereoselective biliary excretion was evaluated in rats with liver injury.

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MATERIALS AND METHODS

Reagents Etdolac was generously supplied by Nippon Shinyaku Co., Ltd. (Kyoto, Japan). Methyl p-hydroxybenzoate and propyl p-hydroxybenzoate, internal standards for HPLC, and glutamic oxaloacetic transaminase (GOT)-UV test Wako were purchased from Wako Pure Chemical Industries (Osaka, Japan). Carboxymethyl cellulose sodium (CMC) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Twenty percent sodium p-aminohippurate injection and 10% sodium thiosulfate injection were obtained from Daiichi Seiyaku Co., Ltd. (Tokyo) and Banyu Seiyaku Co., Ltd. (Tokyo), respectively. All other chemicals and solvents used were of reagent grade or HPLC quality.

Animals Male Wistar rats (120–250 g, 4–7-weeks old) were used throughout this experiment. On the day before the experiment, the right jugular vein of the animals was cannulated with silicon tubing under anesthesia (pentobarbital, 40 mg/kg, i.p.). The free end of the cannula was filled with heparinized (100 IU/ml) normal saline. Animals had free access to MF diet (Oriental Yeast Co., Ltd.) and water before experiments, unless stated otherwise.

Single i.v. and Oral Administrations to Intact Rat Etdolac (30 mg/kg) dissolved in 0.5% Na2CO3 was injected into the jugular vein. Blood samples (0.2 ml) were collected periodically from the jugular vein into a heparinized syringe 0.25–26 h after dosing. After sampling, withdrawn blood was replaced by the injection of an equal volume of heparinized saline. Etdolac (30 mg/kg) suspended in 0.5% CMC solution was administered orally to intact rats previously fasted for 12 h. Blood samples (0.2 ml) were collected periodically after dosing.

Biliary Excretion The common bile duct was cannulated with polyethylene tubing (PE-10) under pentobarbital anesthesia. After wakening, rats were fixed individually in a Bolman cage and etdolac (30 mg/kg)
dissolved in 0.5% Na₂CO₃ was injected into the jugular vein of bile-exteriorized rats (BE-rat). Bile samples were collected periodically through the cannula 0.5—10 h after dosing, and blood samples were withdrawn periodically from the jugular vein of the rat.

Reabsorption of Etodolac in Bile  Under pentobarbital anesthesia, the bile duct of BE rat (donor rat) was connected with the duodenum of the acceptor rat by polyethylene tubing (PE-10). The bile of acceptor rats was eliminated through the tubing. Thus, etodolac in the bile from the donor rat following drug administration (30 mg/kg) was absorbed from the gut of the acceptor rat. Blood samples were collected periodically from the acceptor rat 0.25—26 h after dosing.

Induction of Acute Hepatic Injury and i.v. Administration Male Wistar rats (120—150 g, 4—5-weeks old) were divided into random two groups, each consisting of 4 or 6 rats. The animals were treated for 2 d with daily subcutaneous (s.c.) injections of a mixture of 0.2 ml CCL₄ and 0.2 ml olive oil per 100 g of body weight (CCL₄:rats). Animals of the control group were injected with an equivalent volume of olive oil (control rats). Single i.v. administration of etodolac to both groups was done by the method described above.

Induction of Acute Renal Failure and i.v. Administration Male Wistar rats (190—230 g, 5—6-weeks old) were divided into 2 groups. They were treated for 2 d with daily s.c. injections of 1% uranyl acetate (10 mg/kg) in saline (UA-rats). Animals of the untreated group were subcutaneously administered saline for 2 d (control rats). The next day, the common bladder was cannulated with polyethylene tubing (PE-50) under anesthesia. After wakening, sodium thiocyanate (60 mg/rat) or sodium p-amino hippurate (30 mg/rat) was injected into the jugular vein. Plasma samples (0.25 ml) of rats were taken 0.5 and 1.5 h after dosing, and urinary samples (0.25 ml) were collected over a 1.5-h period after dosing.

Determination of Racemic Etodolac in Plasma and Bile Etodolac in plasma and bile was determined according to the method of Honda et al. with slight modifications. An aliquot (100 µl) of plasma was mixed with 200 µl of internal standard solution (methyl p-hydroxy benzoate, 15 µg/ml, in acetonitrile), followed by shaking and centrifuging. A 200-µl portion of the upper layer was filtered through a membrane filter (0.45 µm, Chromatodisk, GL Sciences Inc. Tokyo, Japan), and then 5—40 µl of the filtrate was injected onto a reversed-phase Inertsil ODS C18 column (4.6 x 250 mm, GL Sciences), using a Shimadzu liquid chromatograph (model LC-6AD, Kyoto, Japan) equipped with an ultraviolet detector (model SPD-10A). The mobile phase was 0.1 M sodium phosphate buffer (pH 7.4)—acetonitrile (68:32, v/v). The flow rate was 1.0 ml/min and the detection was at 225 nm.

The bile sample (10 µl) was diluted with water. The conjugated etodolac in the diluted sample was hydrolyzed with 1 M NaOH at room temperature for 30 min, and then neutralized with 0.6 M H₂SO₄. An aliquot of the hydrolyzed solution was mixed with internal standard solution (n-propyl p-hydroxy benzoate, 10 µg/ml, in methanol, 200 µl), 0.1 N HCl (2 ml) and ethyl acetate (3 ml). The mixture was shaken and then centrifuged. The ethyl acetate layer was evaporated under reduced pressure. The residue dissolved in a small amount of acetonitrile was injected onto the column. The HPLC conditions were the same as described above. Unchanged drug in the bile was determined after direct extraction with isoctane–isopropanol (95:5, v/v) from the bile.

Determination of Etodolac Enantiomers Concentrations of etodolac enantiomers in bile were determined by the method described by Jamali et al. after hydrolysis of conjugates, with slight modifications. Naproxen as an internal standard, a normal-phase analytical column (4.6 x 250 mm) containing Chemcosorb 7CN (5 µm, Chemco Scientific Co., Ltd., Osaka, Japan) and the mobile phase consisting of hexane–ethyl acetate–isopropanol (90:10:0.1, v/v) were used in this experiment. Calibration curves prepared using rat bile yielded excellent linearity ($r^2 > 0.999$) between the peak area ratios and corresponding rat bile concentrations of etodolac. The detection limit was 0.5 µg/ml.

Assay of Hepatic Enzyme Activity The hepatic 9000 x g supernatant was prepared by the usual method from the CCL₄- and control rats. Aniline hydroxylase activity and the concentrations of cytochrome P-450 (P-450) in the 9000 x g supernatant were determined by methods reported by Schenken et al. and Omura and Sato, respectively. GOT activity in plasma was measured using the GOT-UV test Wako.

Analysis of Renal Function Sodium thiosulfate and sodium p-aminohippurate in plasma and urine were determined by the method of Brun. Blood urea nitrogen (BUN) and creatinine in plasma were determined by the diazotymonoxyde method and the Folin–Wu method, respectively. Glomerular flow rate (GFR) and renal plasma flow (RPF) were calculated using the data mentioned above:

$$GFR(\text{ml/min}) = \frac{U \cdot V}{P}$$

$$RPF(\text{ml/min}) = \frac{U \cdot V}{P}$$

where $P$ is the plasma concentration of sodium thiosulfate or p-aminohippurate, $U$ denotes their urinary levels and $V$ is the urine volume.

Pharmacokinetic Model for Enterohepatic Circulation The proposed pharmacokinetic model was developed with reference to the compartment model. A portion of the drug distributed in the central compartment (C₁) after i.v. administration is secreted into the biliary compartment (b), which is the bile fraction in the liver. This bile, excreted into the duodenum, is transferred to the gastrointestinal compartment (g) and the drug generated from the conjugate after hydrolysis is reabsorbed. The model in Fig. 1 can be expressed mathematically with the following differential equations:

$$\frac{dc_1}{dt} = -(k_{a} + k_{b} + k_{C1} + k_{pC}) c_1$$

$$\frac{dc_2}{dt} = -(k_{a} + k_{b} + k_{C2} + k_{pC}) c_2 + k_{tr} X_{g}/V_{c}$$

$$\frac{dc_{pC}}{dt} = k_{apC} (c_1 - k_{PC} c_1)$$

$$\frac{dc_{a}}{dt} = k_{b} (c_2 - k_{PC} c_2)$$
\[
\frac{dX_b}{dt} = k_{cp} \cdot C_e \cdot V_e \cdot k_{bi} \cdot X_b
\]
\[
\frac{dX_e}{dt} = k_e \cdot X_e - k_i \cdot X_e
\]

where \(X_b\) and \(X_e\) are the amount of drug in the biliary compartment and in the gastrointestinal compartment, respectively; \(k_{cp}\) and \(k_{pe}\) represent the distribution rate constants; \(k_i\) and \(k_e\) are the first-order rate constants of the respective steps. \(V_e\) denotes the distribution volume in the central compartment.

**Data Analysis** Pharmacokinetic parameters were calculated using the nonlinear least squares regression program, MULTI.\(^{15}\) The plasma concentration–time data after i.v. administration were fitted to the equation:

\[
C_t = Ae^{-\alpha t} + Be^{-\beta t}
\]

where \(C_t\) is the drug concentration at time \(t\), and \(A, \alpha, B\) and \(\beta\) are the biexponential equation constants. \(AUC\) was determined by the trapezoidal method. The area under the first moment curve (AUUMC), the mean residence time (MRT) and \(V_{des}\) were calculated by the following equations:

\[
AUUMC = \int_{0}^{\infty} t \cdot Cdt
\]

\[
MRT = \frac{AUUMC}{AUC}
\]

\[
V_{des} = \frac{D \cdot AUUMC}{(AUC)^2}
\]

The means of all data are presented with their standard deviation (mean ± S.D.). Statistical analysis was performed using a non-paired Student's \(t\)-test, and a \(p\) value of \(\leq 0.05\) was considered to be significant.

**RESULTS AND DISCUSSION**

**Plasma Concentration of Etdolac in Intact and BE Rats**

Figure 2 shows the plasma concentrations of etdolac after i.v. injection (30 mg/kg) in intact and BE rats. In intact rats, a slow elimination (\(\beta, 0.061 \text{ h}^{-1}\)) and a peak indicative of extensive enterohepatic circulation were seen in plasma time courses of etdolac. The plasma concentrations in BE rats followed typical two-compartment kinetics. The time courses in BE rats nearly coincided with those in the control rats for the first 60 min, but thereafter the decline of the former was considerably faster (\(\beta, 0.176 \text{ h}^{-1}\)) than in the latter. The pharmacokinetic parameters are shown in Table 1. The total clearance (\(Cl_{tot}\), 110.5 ± 53.6 ml/h/kg) in BE rats was larger than that (52.7 ± 18.9 ml/h/kg) in the control rats, with significant difference in \(V_{des}\) values.

Plasma concentrations after oral administration were also measured. A secondary peak in the drug concentrations was clearly recognized about 7 h after dosing, and the elimination (\(\beta, 0.037 \text{ h}^{-1}\)) of the drug in plasma was slower than that (0.061 h\(^{-1}\)) after i.v. dosing. Thus, these results indicated that this drug was subjected to extensive enterohepatic circulation in rats and that its slow elimination was mainly due to this circulation.

**Biliary Excretion after i.v. Administration** The biliary excretion of etdolac after i.v. administration is shown in Fig. 3A. Total biliary excretion of the drug during 10 h after dosing was about 45% of the dose. Ninety eight percent of total etdolac excreted in the bile during the 10 h was secreted within 4 h after administration, and almost all of it excreted in bile was glucuronides, as shown by the negligible amount (2%) of the free drug remaining.

**Plasma Concentrations of Etdolac in Bile Duct-Linked (Bile-Linked) Rat** The plasma concentrations of etdolac in an acceptor rat (bile-linked rat) which received the bile of the donor rat dosed intravenously this drug are shown in Fig. 3B. The peak concentration was observed 6—7 h after dosing. Peak time of the plasma concentration was well consistent with the secondary peak in plasma levels after oral dosing in intact rats. The relatively delayed
peak (Fig. 3B) might be ascribed to the slow absorption of the drug in bile, probably due to the slow hydrolysis of the conjugates in intestine. The S-enantiomer was predominant in the plasma of the bile-linked rat, agreeing well with the data that the S-enantiomer has a greater biliary clearance than R-enantiomer and exhibits extensive enterohepatic recycling.1,5)

Pharmacokinetic Analysis of Enterohepatic Circulation
Pharmacokinetic data for etodolac in BE and bile-linked rats were analyzed in terms of the model shown in Fig. 1. The model was adapted to the observed plasma concentrations after i.v. administration to BE rats. The solid lines in Fig. 4 represent the simulated curves calculated by the model. Approximate agreement between the simulation curve and the observed data was obtained as shown in the figure, except for the peak levels of the simulation curve for the bile-linked rat, in which the peak concentration of the drug observed was higher than that simulated by the model. The discrepancy in the simulated and observed data may be partly ascribable to the slightly delayed excretion of bile through the polyethylene tubing used for the connection with the acceptor rat in the bile-linked rat. Consequently, this multicompartment model was approximately able to describe the time course of etodolac levels in the enterohepatic circulation.

The model-independent parameters of etodolac disposition in control and bile-linked rats are shown in Table 2. The $k_o$ value for bile-linked rats was 0.27 ± 0.04 h⁻¹, demonstrating the large excretion of the conjugates into bile.

Table 2. Model-Independent Pharmacokinetic Parameters of Etodolac Disposition in Control and Bile-Linked Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Bile-linked</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_c$ (ml/kg)</td>
<td>227 ± 47.7</td>
<td>223 ± 8.5</td>
</tr>
<tr>
<td>$k_{op}$ (h⁻¹)</td>
<td>0.64 ± 0.32</td>
<td>0.61 ± 0.09</td>
</tr>
<tr>
<td>$k_{on}$ (h⁻¹)</td>
<td>0.52 ± 0.32</td>
<td>0.49 ± 0.10</td>
</tr>
<tr>
<td>$k_{o}$ (h⁻¹)</td>
<td>0.50 ± 0.27</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>$k_{c}$ (h⁻¹)</td>
<td>0.17 ± 0.04</td>
<td>0.55 ± 0.11</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. $a$) $k_o$ plus $k_c$. 

Plasma Concentrations of Etodolac in CCl₄- and UA-Rats

To confirm the impairment of hepatic and renal function, the hepatic enzyme activities and renal parameters were measured after treatment with CCl₄ and uranil acetate for 2d, respectively. The enzyme activities and the parameters are shown in Table 3. Hepatic failure induced by CCl₄ dramatically decreased the P-450 content and aniline hydroxylase activity and increased the GOT compared with those of the control rats; this clearly demonstrated that the hepatic failure was induced by
Table 3. Comparison of Liver Metabolic Enzyme and Renal Function in Control, CCl₄- and UA-Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control rat</th>
<th>CCl₄-rat</th>
<th>UA-rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-450 (nmol/mg protein)</td>
<td>0.45 ± 0.08</td>
<td>0.17 ± 0.03⁴</td>
<td>—</td>
</tr>
<tr>
<td>Aniline hydroxylase (nmol/mg protein/min)</td>
<td>0.59 ± 0.10</td>
<td>0.07 ± 0.05⁴</td>
<td>—</td>
</tr>
<tr>
<td>GOT (IU)</td>
<td>50.0 ± 8.7</td>
<td>270.3 ± 74.9⁴</td>
<td>—</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>3.26 ± 0.43</td>
<td>0.25 ± 0.17⁴</td>
<td>—</td>
</tr>
<tr>
<td>RPF (ml/min)</td>
<td>6.53 ± 1.80</td>
<td>0.72 ± 0.25⁴</td>
<td>—</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>26.0 ± 16.4</td>
<td>114.5 ± 17.7⁴</td>
<td>—</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>3.25 ± 0.58</td>
<td>4.53 ± 1.13</td>
<td>—</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. (n = 4). ⁴ p < 0.01 compared with the control.

CCl₄. On the other hand, GFR and RPF were significantly decreased, while BUN was drastically increased by uranyl acetate compared with those in the control rats. Consequently, the incidence of acute renal failure was demonstrated in the animals treated with uranyl acetate.

Figure 5 shows the mean plasma concentration profiles of etodolac in the control, CCl₄- and UA-rats. The elimination during the first 1 h was not influenced by the hepatic or renal failure, but thereafter the elimination was significantly retarded in rats with both diseases and high plasma levels of the drug were observed over longer periods. In animals with hepatic failure, the plasma concentrations were appreciably higher than those in UA-rat; suggesting that the effect of hepatic failure on the rate of elimination was more severe than that of renal disease. The kinetic parameters for these rats are shown in Table 4. In CCl₄- and UA-rats, the AUC was 2.5 and 2 times larger, respectively, and Cltot was a half and two-thirds, respectively, compared with those in the control rats, without significant changes in the β values. Thus, the decrease in Cltot of CCl₄- and UA-rats was mainly due to the decrease in Va in based on the failure of tissues, resulting in the high plasma concentrations of etodolac. The higher plasma levels observed in CCl₄-rats was partly due to the decrease in the metabolic conversion of etodolac to the oxidative products, which are the hydroxy derivatives,⁵ leading to the decrease in elimination of the unchanged drug from plasma. The relatively high plasma concentrations in UA-rats might also be explained by the decreased tubular secretion (active transport of organic anions).

Biliary Excretion of Enantiomers in Acute Hepatic Disease The biliary excretion of etodolac enantiomers was measured in relation to hepatic failure. The cumulative excreted amount of the enantiomers after administration of racemic etodolac to rats in the control and CCl₄-groups is shown in Fig. 6. The excreted bile contained 71.0 ± 13.8% S-enantiomer and 28.6 ± 9.1% R-enantiomer of the dose of racemic drug in the control rats. These values agreed well with data reported by Brocks and Jamali who showed the complete recovery of S-enantiomer in bile, but only 30% of R-enantiomer via this route of elimination.⁴

Of particular interest was that the biliary excretion (R-enantiomer 28.6 ± 6.9%; S-enantiomer 77.7 ± 9.4%) of the enantiomers in rats with the acute hepatic disease was not significantly different from that of the control rats. This indicates that the acute hepatic disease apparently did not influence the total biliary excretion of R- and S-enantiomer or the micosomal glucuronidation activity.
although treatment with CCl₄ would decrease the oxidative metabolism and, to some degree, glucuronidation. Additionally, when the intact (free) form in the bile was measured, no significant difference in amount was observed between the control and CCl₄-rats (intact form, about 2% of the amount excreted in bile). These results were in keeping with the data reported by Desmond et al. that hepatic glucuronyl transferase activity was preserved when mixed-function oxidase activity was impaired by CCl₄.¹⁷ The preservation of conjugation in patients with liver disease was also reported.¹⁸

The possible explanation for the preservation is that mixed-function oxidase enzymes are located on the surface of the hepatic cell membrane, while glucuronyl transferase enzymes are distributed deeper within the membrane.¹⁹ As a consequence, under normal conditions, only a relatively small proportion of glucuronyl transferase enzymes would interact with substrate. In contrast, after CCl₄ pretreatment the disruption of the membrane would result in increased activity of the enzymes, and apparently high activity of transferase was preserved. Bock et al. demonstrated similar trends with 1-naphthol glucuronidation.²⁰ Consequently, CCl₄-induced liver injury had no effect on the enterohepatic circulation of etodolac in rats.

In conclusion, racemic etodolac was subjected to extensive enterohepatic circulation in rats. Total biliary excretion of the drug during 10 h after dosing was about 45% of the dose. The pharmacokinetic model for the enterohepatic circulation could well describe the plasma concentrations of the drug reabsorbed in the gastrointestinal compartment. Hepatic and renal failure markedly retarded the elimination. However, the biliary excretion of etodolac enantiomers, in the form of glucuronides, was not affected by the hepatic disease.

REFERENCES