Stereoselective Pharmacokinetics and Chiral Inversion of Ibuprofen in Adjuvant-induced Arthritic Rats

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ABSTRACT

2-Arylpropionic acid (2-APA) nonsteroidal anti-inflammatory drugs are commonly used in racemic mixtures (rac) for clinical use. 2-APA undergoes unidirectional chiral inversion of the in vivo inactive R-enantiomer to the active S-enantiomer. Inflammation causes the reduction of metabolic activities of drug-metabolizing enzymes such as cytochrome P450 (P450) and UDP-glucuronosyltransferase. However, it is unclear whether inflammation affects the stereoselective pharmacokinetics and chiral inversion of 2-APA such as ibuprofen (IB). We examined the effects of inflammation on the pharmacokinetics of R-IB and S-IB after intravenous administration of rac-IB, R-IB, and S-IB to adjuvant-induced arthritic (AA) rats, an animal model of inflammation. The plasma protein binding of rac-IB, glucuronidation activities for R-IB and S-IB, and P450 contents of liver microsomes in AA rats were determined. Total clearance (Cl tot) of IB significantly increased in AA rats, although the glucuronidation activities for IB, and P450 contents of liver microsomes decreased in AA rats. We presumed that the increased Cl tot of IB in AA rats was caused by the elevated plasma unbound fraction of IB due to decreased plasma albumin levels in AA rats. Notably, Cl tot of R-IB but not S-IB significantly increased in AA rats after intravenous administration of rac-IB. These results suggested that AA could affect drug efficacies after stereoselective changes in the pharmacokinetics of R-IB and S-IB.

Introduction

The 2-arylpropionic acid (2-APA) nonsteroidal anti-inflammatory drugs, with the exception of naproxen, are commonly used in racemic mixtures (rac) for clinical use. The S-enantiomer of 2-APA confers therapeutic effects via prostaglandin synthesis inhibition and causes adverse effects such as gastrointestinal irritation (Brune et al., 1992). 2-APA undergoes unidirectional chiral inversion of the in vivo inactive R-enantiomer to the active S-enantiomer, which appears to be species and compound dependent (Caldwell et al., 1988; Baillie et al., 1989; Knijnicki et al., 1989; Chen et al., 1990; Müller et al., 1990; Ahn et al., 1991; Chen et al., 1991; Rudy et al., 1991). Stereoselective pharmacokinetic studies of 2-APA have been performed in rodents and humans (Abas and Meffin, 1987; Foster et al., 1987; Fantifer et al., 1988; Jamali et al., 1988; Pedrazzini et al., 1988; Jamali and Brooks, 1990; Brooks and Jamali, 1994; Davies, 1995; Castro et al., 2001).

The liver is susceptible to inflammation such as viral and drug-induced hepatitis and is the primary organ for metabolism of xenobiotics and endogenous substrates. We previously demonstrated alterations in expression and activity of drug-metabolizing enzymes and transporters in inflammation using mice with collagen-induced arthritis (Kawase et al., 2007) and rats with adjuvant-induced arthritis (AA) (Uno et al., 2007,2009).

AA rats have been used as a model of rheumatoid arthritis for the development of anti-inflammatory medicines because they exhibit systemic inflammatory disease with changes to bone and cartilage similar to those observed in humans with rheumatoid arthritis (Williams, 1992). Increases in inflammatory markers such as lactate dehydrogenase, aspartate aminotransferase, alkaline phosphatase, and α1-acid glycoprotein were observed in the serum of AA rats (Kawase et al., 2013). Several reports have shown that the elimination of propranolol (Walker et al., 1986; Picuette-Miller and Jamali, 1995), acebutolol (Picuette-Miller and Jamali, 1992), and cyclosporine (Pollock et al., 1989) from blood is delayed in AA rats. The alterations in drug pharmacokinetics in AA rats are possibly attributed to changes in plasma binding to albumin and impaired metabolism by metabolic enzymes such as cytochrome P450 (P450) and UDP-glucuronosyltransferase (UGT). Most 2-APA drugs are highly bound to plasma albumin and are mainly eliminated by hepatic metabolism. Meunier and Verbeeck (1999a) reported the stereoselective glucuronidation of ketoprofen in AA rats. They showed that AA rats exhibit significant impairment in the in vivo glucuronidation of ketoprofen. Consequently, the disposition of drugs that undergo low hepatic extraction can be especially affected by pathophysiological changes in arthritis. We demonstrated that the total clearance (Cl tot) of flurbiprofen enhanced owing to a remarkable decrease in protein binding despite impaired intrinsic hepatic clearance (Cl inte) (Nagao et al., 2003).

However, there is little information regarding the pharmacokinetics of chiral inversion of 2-APA in arthritis, despite 2-APA being widely administered to patients suffering from arthritis. Ibuprofen (IB) as a model compound of 2-APA undergoes unidirectional chiral inversion of...
the inactive \(R\)-enantiomer to the active \(S\)-enantiomer in rats (Kaiser et al., 1976). We previously demonstrated that the intrinsic chiral inversion rate constant of IB and the metabolic degradation rate constants decreased in AA rats compared with those of control rats in freshly isolated rat hepatocytes (Uno et al., 2008). This in vivo study was carried out to clarify the effects of AA inflammation on the stereo-selective pharmacokinetics of \(R\)-IB and \(S\)-IB and chiral inversion of IB after intravenous administration to rats.

### Materials and Methods

**Ethical Approval of the Study Protocol.** The study protocol was approved by the Committee for the Care and Use of Laboratory Animals of the Faculty of Pharmacy of Kindai University (Osaka, Japan).

**Compounds and Reagents.** \(Rac\)-IB was purchased from Wako Pure Chemicals (Osaka, Japan), \(R\)-IB and \(S\)-IB were purchased from Cayman Chemical Company (Ann Arbor, MI) and Tokyo Kasei (Tokyo, Japan), respectively. Naproxen and etodolac, internal standards for high-performance liquid chromatography (HPLC), were purchased from Sigma Aldrich (St. Louis, MO) and Nippon Shinyaku (Kyoto, Japan), respectively. Rabbit polyclonal anti-acyl-coenzyme A synthetase (ACS1) antibody (Bioss Antibodies, Woburn, MA), rabbit polyclonal anti-2-aryl propionyl-coenzyme A epimerase (APCE) antibody (Cell Signaling Technology, Danvers, MA), and mouse monoclonal anti-\(\beta\)-actin (Akris Antibodies, Herford, Germany). All other chemicals and solvents were of the best purity commercially available or of HPLC grade.

**Preparation of AA Rats.** Female Sprague-Dawley rats (7 weeks old) weighing 150–170 g were purchased from Japan SLC (Shizuoka, Japan). The animals were housed in a temperature-controlled room with free access to standard laboratory food and water. AA rats were prepared according to a previously reported procedure (Kawase et al., 2014). Briefly, 1 g meat-killed *Mycobacterium butyricum* was subcutaneously injected into the right hind footpad and tail base of rats (Difco Laboratories, Detroit, MI) as an adjuvant suspended in Bayol F oil (10 mg/ml). Hind paw volumes were measured using a liquid plethysmometer. Animals that exhibited severe inflammation at local and systemic sites at 21 days (chronic phase) after injection of the adjuvant were studied.

**Animal Experiments.** On the day before drug administration, a cannula (silicone tubing; 0.5 mm inside diameter [ID], 1.0 mm outside diameter [OD]) was implanted in the right jugular vein under pentobarbital anesthesia (40 mg/kg, intraperitoneally). On day 21 after AA induction, the bile ducts of the animals were cannulated with polyethylene tubing (PE-10; 0.28 mm ID, 0.61 mm OD) to avoid possible enterohepatic circulation of IB (Dietzel et al., 1990). Animals received intravenous administration of either \(rac\)-IB (20 mg/kg) or each IB enantiomer (10 mg/kg) through the jugular vein cannula, followed by 0.3 ml of sterile heparinized saline to flush the tubing. Blood samples were collected 1, 5, 10, 20, 30, 45, 60, 90, and 120 minutes after administration and were centrifuged for 10 minutes at 3000 \(g\). Because of the instability of acyl glucuronides at physiologic \(pH\), plasma was transferred to a tube containing 5 \(\mu\)l 17% phosphoric acid. The plasma was immediately frozen on dry ice and stored at \(-80^\circ\text{C}\) until analysis.

**Protein Binding Study.** In vitro filtrated unbound IB at a total concentration of 50 \(\mu\)g/ml was determined by ultrafiltration (MINICENT-10, Tosoh, Tokyo, Japan) of plasma samples from control and AA rats without IB treatment to evaluate the plasma protein binding of IB. In vivo plasma protein binding in control and AA rats was also measured after intravenous administration of \(rac\)-IB (20 mg/kg). Preliminary studies indicated that IB did not bind to the ultrafiltration device. Plasma albumin and total protein concentrations were determined by an automatic analytical device (Olympus AU5200, Olympus, Tokyo, Japan).

**Glucuronidation Activities and P450 Contents in Liver Microsomes.** The UGT activities in rat liver microsomes for IB enantiomers were determined. After incubating 1 nM IB for 10 minutes in the microsomal suspension in 0.1 M Tris-HCl buffer (pH 7.4) including 1 mM glucose-6-phosphate, 10 mM MgCl\(_2\), 0.2% Triton X-100, 2 nM phenyl methyl sulfonil fluoride, 20 mM 1,4-saccharolactone, and 10 mM UDP-glucuronic acid, the concentrations of IB glucuronide (IBG) formed were stereoselectively determined by HPLC. P450 contents in rat liver microsomes were determined by the method described by Omura and Sato (1964).

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**Sample Preparation for HPLC Analysis.** Naproxen (50 \(\mu\)l) in methanol (1 \(\mu\)g/ml) was added as an internal standard to mixtures of 100 \(\mu\)l plasma and 400 \(\mu\)l acetic acid (pH 2.5). After the addition of 2 ml ethyl acetate, the mixture was shaken for 10 minutes and centrifuged at 2000 \(g\) for 5 minutes. The organic layer was transferred to another clean tube and evaporated to dryness using a centrifugal evaporator (Tokyo Rikakikai, Tokyo, Japan) at 35°C. The 20-\(\mu\)l residue dissolved in 100 \(\mu\)l of mobile phase was injected into the HPLC system.

**HPLC Conditions.** HPLC analysis was performed using a conventional ODS column (Cosmosil 5C18-AR-II, 4.6 \times 250 mm, 5 \(\mu\)m, Nacalai Tesque, Kyoto, Japan) for \(rac\)-IB and a chiral column (Chiral OD-R, 4.6 \times 150 mm, 5 \(\mu\)m, Daicel Chemical, Tokyo, Japan) for \(R\)-IB, \(S\)-IB, and \(S\)-IBG, using a Shimadzu HPLC system equipped with an ultraviolet detector (220 nm for IB assay, 232 nm for IBG assay). The mobile phases (0.2 M phosphate buffer, pH 2.0/acetonitrile (67.5:32.5, v/v) for IB assay, and 0.05 M phosphate buffer including 2 mM tetra-n-butylammonium hydrogen sulfate, pH 5.5/acetonitrile (65:35, v/v) for IBG) were pumped at a flow rate of 1 ml/min.

**Pharmacokinetic Analysis.** Plasma concentration data for IB were fitted to the mass balance equation for a conventional two-compartment open model with unidirectional inversion from \(R\)-IB to \(S\)-IB (Fig. 1) (Knihinicki et al., 1990). We tried to one- and two-compartment model including chiral inversion. The Akaike’s information criterion of two-compartment model was smaller than that...
of one-compartment model. Pharmacokinetic parameters were obtained by the nonlinear least square method using WinNonlin software (Pharsight, Mountain View, CA). Plasma concentration-time profiles of S-IB after intravenous administration of S-IB were fitted to the following equations:

\[
\begin{align*}
V_1(\text{s}) \cdot (dC_{1}(\text{s})/dt) &= -(k_{10(\text{s})} + k_{21(\text{s})}) \cdot V_1(\text{s}) \cdot C_{1(\text{s})} + k_{21(\text{s})} \cdot X_{2(\text{s})} \\
\frac{dX_{2(\text{s})}}{dt} &= k_{21(\text{s})} \cdot X_{1(\text{s})} - k_{21(\text{s})} \cdot X_{2(\text{s})}
\end{align*}
\]

where \(C_{1(\text{s})}\) is the plasma concentration of S-IB in the central compartment; \(X_{1(\text{s})}\) and \(X_{2(\text{s})}\) are the amounts of S-IB in the central and peripheral compartments, respectively; \(V_{1(\text{s})}\) is the volume of distribution of the central compartment; \(k_{10(\text{s})}\), \(k_{21(\text{s})}\), and \(k_{12(\text{s})}\) are the first-order rate constants as shown in Fig. 1.

Determination of Protein Levels by Western Blot. Hepatic microsomes of control and AA rats were prepared according to a previously reported procedure (Iwaki et al., 1995; Nozaki et al., 2007). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed using 7.5% e-Pagell (Atto, Tokyo, Japan) and 50 μg microsome per well. Resolved proteins were transferred onto Hybrid-P polyvinylidene difluoride membranes (GE Healthcare, Milwaukee, WI). Immunoreactive ACS1, APCE, and β-actin were detected using antibodies and an ECL Prime Western Blotting Detection system (GE Healthcare).

### Table 1
Noncompartmental pharmacokinetic parameters of each enantiomer after intravenous administration of rac-IB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>S-IB</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_1) (ml/kg)</td>
<td>63.1 ± 4.4</td>
<td>67.3 ± 9.9</td>
<td>86.0 ± 34.2</td>
</tr>
<tr>
<td>(V_2) (ml/kg)</td>
<td>56.4 ± 24.6</td>
<td>56.0 ± 23.2</td>
<td>80.3 ± 28.4</td>
</tr>
<tr>
<td>(CL_{hep}) (ml/min/kg)</td>
<td>4.01 ± 0.40</td>
<td>1.46 ± 0.40</td>
<td>6.16 ± 1.11</td>
</tr>
<tr>
<td>(CL_{tot}) (ml/min/kg)</td>
<td>2.75 ± 0.39</td>
<td>1.46 ± 0.40</td>
<td>2.19 ± 0.55</td>
</tr>
<tr>
<td>(k_{021}) (min(^{-1}))</td>
<td>0.20 ± 0.001</td>
<td>–</td>
<td>0.024 ± 0.007</td>
</tr>
<tr>
<td>(k_{12}) (min(^{-1}))</td>
<td>0.072 ± 0.031</td>
<td>0.070 ± 0.035</td>
<td>0.059 ± 0.030</td>
</tr>
<tr>
<td>(k_{21}) (min(^{-1}))</td>
<td>0.074 ± 0.024</td>
<td>0.062 ± 0.020</td>
<td>0.076 ± 0.019</td>
</tr>
<tr>
<td>(k_{10}) (min(^{-1}))</td>
<td>0.064 ± 0.007</td>
<td>0.021 ± 0.003</td>
<td>0.076 ± 0.017</td>
</tr>
<tr>
<td>(k_{20}) (min(^{-1}))</td>
<td>0.044 ± 0.007</td>
<td>–</td>
<td>0.052 ± 0.022</td>
</tr>
</tbody>
</table>

* \(p < 0.05\) compared with controls.  
* \(p < 0.05\) compared with its antipode.

### Table 2
Compartmental pharmacokinetic parameters of each enantiomer after intravenous administration of rac-IB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>S-IB</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_1) (ml/kg)</td>
<td>35.7 ± 6.8</td>
<td>89.5 ± 37.1</td>
<td>30.7 ± 6.5</td>
</tr>
<tr>
<td>(CL_{hep}) (ml/min/kg)</td>
<td>3.70 ± 0.14</td>
<td>0.80 ± 0.27</td>
<td>5.40 ± 0.71</td>
</tr>
<tr>
<td>(MRT) (min)</td>
<td>32.7 ± 3.9</td>
<td>127 ± 51.9</td>
<td>30.3 ± 5.7</td>
</tr>
<tr>
<td>(V_d) (ml/kg)</td>
<td>120 ± 18</td>
<td>89 ± 10</td>
<td>165 ± 38</td>
</tr>
<tr>
<td>(AUC_{0-\infty}) (μg min/ml)</td>
<td>2720 ± 103</td>
<td>14031 ± 4496</td>
<td>1862 ± 230</td>
</tr>
</tbody>
</table>

* \(p < 0.05\) compared with controls.  
* \(p < 0.05\) compared with its antipode.  

The em dashes indicate no value because of unidirectional chiral inversion of IB.
The compartment (V2) of large. The CLtot of each enantiomer was approximately 1.5 times between enantiomers, although the volumes of R clearance (CLtot) and shorter mean residence time were observed from the results in Fig. 2 are summarized in Table 1. The stereoselective controls. The noncompartmental pharmacokinetic parameters estimated from rats were determined (Fig. 2). The plasma distribution volume of the central compartment (V1) and the peripheral compartments model for each enantiomer of IB are summarized in Table 2. The dis-

**Results**

The plasma concentration-time profiles of IB enantiomers after intravenous bolus injection of rac-IB (20 mg/kg) to control and AA rats were determined (Fig. 2). The plasma R-IB in both control and AA rats was rapidly eliminated. The plasma concentrations of both enantiomers in AA rats were slightly lower than corresponding antipodes in the controls. The noncompartmental pharmacokinetic parameters estimated from the results in Fig. 2 are summarized in Table 1. The stereoselective pharmacokinetics of IB were observed; for R-IB, relatively higher total clearance (CLtot) and shorter mean residence time were observed compared with those of S-IB in both control and AA rats. There were no significant differences in distribution volumes at steady state (Vdss) between enantiomers, although the volumes of R-IB tended to be large. The CLtot of each enantiomer was approximately 1.5 times increased in AA rats compared with that of control rats, although the increase in CLtot of S-IB in AA rats was not significant.

To elucidate the effect of AA on the chiral inversion of R-IB to the S-antipode, compartment model analysis including the inversion process was performed. The pharmacokinetic parameters obtained from the model for each enantiomer of IB are summarized in Table 2. The distribution volume of the central compartment (V1) and the peripheral compartment (V2) of R-IB and S-IB in AA rats were slightly higher compared with those of the controls. There were no significant differences in metabolic clearance (CLMET) between enantiomers in either control or AA rats. However, the CLmet of R-IB was significantly higher than that of S-IB owing to the inversion of R-IB to S-IB. The inversion clearance (CLRS) and CLMET of R-IB increased in AA rats; the CLtot, CLRS, and CLMET values were approximately 1.5 times higher in AA rats. On the other hand, there were no significant differences in CLtot and CLMET of S-IB between control and AA rats. Slight differences in kRS, k12, k21, k10, and k02 were observed between control and AA rats. The inversion rate constants (kRS) for R-IB in control and AA rats were 0.044 and 0.052 minute⁻¹, respectively, which accounted for approximately 70% of k10.

To further clarify the effects of AA on the pharmacokinetics of each enantiomer, plasma concentration profiles of the IB enantiomers after the intravenous administration of either R-IB or S-IB were examined (Fig. 3). R-IB was not detected in plasma after S-IB administration, indicating that no or negligible inversion from S-IB to R-IB occurred in rats. S-IB was observed in plasma after R-IB administration, demonstrating the in vivo inversion of R-IB to S-IB. Plasma concentrations of S-IB increased up to approximately 20 minutes after the administration of R-IB. Subsequently, both R-IB and S-IB concentrations decreased and the concentrations of S-IB were higher than those of R-IB from approximately 30 minutes onward. The noncompartmental and compartmental pharmacokinetic parameters of R-IB and S-IB after intravenous administration of each enantiomer were estimated (Tables 3 and 4). The T1/2 and Vdss for R-IB and S-IB did not change between control and AA rats. The CLtot of R-IB and S-IB in AA rats significantly

**TABLE 3**

Noncompartmental pharmacokinetic parameters of each enantiomer after intravenous administration of R-IB or S-IB

Results are expressed as the mean ± S.D. (n = 3).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1/2 (min)</td>
<td>33.9 ± 4.5</td>
<td>77.8 ± 24.4</td>
</tr>
<tr>
<td>CLtot (ml/kg)</td>
<td>4.20 ± 0.23</td>
<td>–</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>32.8 ± 5.4</td>
<td>–</td>
</tr>
<tr>
<td>Vdss (ml/kg)</td>
<td>137 ± 18</td>
<td>–</td>
</tr>
<tr>
<td>AUC (μg·min/ml)</td>
<td>2386 ± 129</td>
<td>3549 ± 474</td>
</tr>
</tbody>
</table>

aP < 0.05 compared with controls.
bP < 0.05 compared with its antipode.

The em dashes indicate no value because of unidirectional chiral inversion of IB.
Compartmental pharmacokinetic parameters of each enantiomer after intravenous administration of R-IB or S-IB

Results are expressed as the mean ± S.D. (n = 3).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>S-IB</th>
<th>AA</th>
<th>R-IB</th>
<th>S-IB</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1 (ml/kg)</td>
<td>90.1 ± 20.0</td>
<td>67.2 ± 9.8</td>
<td>113 ± 38</td>
<td>99.3 ± 18.3</td>
<td></td>
</tr>
<tr>
<td>V2 (ml/kg)</td>
<td>78.5 ± 29.2</td>
<td>93.4 ± 72.2</td>
<td>125 ± 52</td>
<td>112 ± 69</td>
<td></td>
</tr>
<tr>
<td>CLmax (ml/min/kg)</td>
<td>4.28 ± 0.19</td>
<td>1.26 ± 0.12b</td>
<td>6.87 ± 1.05c</td>
<td>2.83 ± 0.92d</td>
<td></td>
</tr>
<tr>
<td>CLmicrosomal (ml/min/kg)</td>
<td>2.21 ± 0.53</td>
<td>-</td>
<td>3.81 ± 0.42</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CLMET (ml/min/kg)</td>
<td>2.07 ± 0.32</td>
<td>1.26 ± 0.12b</td>
<td>3.05 ± 0.42</td>
<td>2.83 ± 0.92</td>
<td></td>
</tr>
<tr>
<td>k0,IB</td>
<td>0.023 ± 0.003</td>
<td>-</td>
<td>0.029 ± 0.009</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>k1 (min⁻¹)</td>
<td>0.026 ± 0.018</td>
<td>0.056 ± 0.030</td>
<td>0.029 ± 0.010</td>
<td>0.058 ± 0.020</td>
<td></td>
</tr>
<tr>
<td>k1 (min⁻¹)</td>
<td>0.031 ± 0.018</td>
<td>0.046 ± 0.020</td>
<td>0.033 ± 0.013</td>
<td>0.050 ± 0.017</td>
<td></td>
</tr>
<tr>
<td>k0,1</td>
<td>0.048 ± 0.008</td>
<td>0.019 ± 0.004a</td>
<td>0.060 ± 0.008</td>
<td>0.028 ± 0.006b</td>
<td></td>
</tr>
<tr>
<td>kbg (min⁻¹)</td>
<td>0.026 ± 0.011</td>
<td>-</td>
<td>0.032 ± 0.003</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 compared with controls.

*p < 0.05 compared with its antipode.

The em dashes indicate no value because of unidirectional chiral inversion of IB.

Discussion

In arthritis, P450 protein levels are decreased (Toda et al., 1994; Meunier and Verbeeck, 1999a; Kawase et al., 2013). In AA rats, used as a model of rheumatic arthritis, the pharmacokinetics of various drugs are affected, owing to the alterations in P450 activities and plasma protein levels (Walker et al., 1986; Pollock et al., 1989; Piquette-Miller and Jamali, 1992, 1995; Meunier and Verbeeck, 1999b). For example, the CLtot of the unbound form of ketoprofen significantly decreased in AA rats, whereas that of total (bound and unbound) ketoprofen was unchanged (Meunier and Verbeeck, 1999b). We also reported that CLtot of flurbiprofen increased approximately twofold in AA rats (Nagao et al., 2003). In the present study, we examined the effects of AA on the stereoselective pharmacokinetics, especially on chiral inversion, of IB in rats.

In AA rats, CLtot of IB significantly increased, although the glucuronidation activities for IB and P450 contents in liver microsomes decreased to approximately 50% and 70%, respectively; it is unclear whether AA affects the protein or activity of UGT and P450 isoforms. Probably, the increased CLtot of IB was caused by the elevated levels of fub of IB due to the decreased plasma albumin levels in AA rats (Tables 7 and 8).

Metabolic enzyme activities and plasma protein binding are the most important determinants of the pharmacokinetics of 2-APA. To clarify whether AA affected metabolic enzyme activities, we measured the glucuronidation activities and P450 contents of rat liver microsomes (Table 6). The glucuronidation activities for S-IB were approximately three to four times higher than those for R-IB in control and AA rats. AA induction resulted in a significant decrease in glucuronidation activities and P450 contents for both R-IB and S-IB, suggesting that phase I and II metabolisms for IB were reduced in AA rats. Total protein and albumin concentrations and the plasma protein binding of R-IB and S-IB in control and AA rats were measured (Tables 7 and 8).

The CLRS of IB in both control and AA rats increased approximately 2.5 times compared with those in controls, although fub of S-IB was significantly greater than that of R-IB in both control and AA rats.

TABLE 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>S-IB</th>
<th>AA</th>
<th>R-IB</th>
<th>S-IB</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F_t )</td>
<td>0.47 ± 0.07</td>
<td>0.51 ± 0.13</td>
<td>0.52 ± 0.12</td>
<td>0.51 ± 0.08</td>
<td>0.53 ± 0.18</td>
</tr>
<tr>
<td>( T_{1/2inv} ) (min)</td>
<td>-</td>
<td>15.9 ± 4.4</td>
<td>29.7 ± 10.5</td>
<td>-</td>
<td>14.1 ± 3.4</td>
</tr>
</tbody>
</table>

\( aF_{AUC} = \frac{AUC_{R-IB}}{AUC_{S-IB}} \)

\( bF_{AUC} = \frac{F_{AUC}}{\frac{AUC_{R-IB}}{AUC_{S-IB}}} \)

\( cF_{COMP} = \frac{k_{u,IB}}{F_{AUC} + k_{u,IB}} \)

The em dashes indicate no value because of unidirectional chiral inversion of IB.
levels in AA rats leading to altered protein binding of drugs affect the pharmacokinetics of propranolol and flurbiprofen, which extensively bind to plasma protein under inflammatory conditions (Kawase et al., 2013). In humans, IB is also extensively (>98%) bound to albumin at therapeutic concentrations (Davies, 1998) and $f_u$ of S-IB is twofold higher compared with that of R-IB, similar to the results in Table 8 (Evans et al., 1990; Tan et al., 2002). It is important to clarify the effects of AA on plasma levels of S-IB, because S-IB is involved in the expression of drug actions. In AA rats, decreased plasma levels of S-IB were observed after intravenous injection of rac-IB, R-IB, or S-IB compared with control rats (Figs. 2 and 3) without change of $F_{i}$ and $T_{1/2INV}$, suggesting that drug actions of IB may be reduced in AA rats.

It is known that nonsteroidal anti-inflammatory drugs such as IB are conjugated in the liver to acyl glucuronides and excreted in bile (Mills et al., 1973). The efficiency of glucuronidation for R-IB or S-IB was suppressed in AA rats to 51% or 44% of control rats, respectively (Table 6). These results agreed with the report about ketoprofen by Meunier et al. (Meunier and Verbeeck, 1999b). The P450 contents in liver microsomes of AA rats significantly decreased by approximately 70% compared with that of control rats. Because most 2-APA including IB have relatively low levels of hepatic extraction, CL$_{TOT}$ of these drugs depends on both $f_u$ and CL$_{INT}$. IB is mainly metabolized by P450 and UGT (Hamman et al., 1997; Buchheit et al., 2011). The CL$_{INT}$ of AA rats could be decreased to approximately half the value of control rats because CL$_{INT}$ reflects the ability to eliminate the metabolized drug from the liver. Therefore, the increase of approximately 1.5 times in CL$_{MET}$ of IB in AA rats after rac-IB administration could be a response to changes in $f_u$ and CL$_{INT}$, that is, in vivo plasma $f_u$ of IB approximately 2.5 times (Table 8) and CL$_{INT}$ decreased by approximately half compared with that in control rats. Meunier and Verbeeck (1999b) showed that CL$_{TOT}$ of ketoprofen in AA rats did not significantly change. This result was interpreted as the approximately doubled $f_u$ levels of ketoprofen negated the effect of decreased CL$_{INT}$ levels. The markedly elevated CL$_{TOT}$ of flurbiprofen in AA rats was assumed to be induced by the relatively higher protein-binding ratio of nonsteroidal anti-inflammatory drugs (Borgâ and Borgâ, 1997; Nagao et al., 2003). After intravenous administration of R-IB or S-IB, the effects of AA on CL$_{TOT}$, CL$_{RS}$, and CL$_{MET}$ of S-IB were larger compared with those of R-IB, although rac-IB exhibited similar effects of AA on CL$_{TOT}$ and CL$_{MET}$ between R-IB and S-IB (Fig. 5). As these results demonstrate, there were some differences between the pharmacokinetics of each enantiomer of IB after intravenous administration of rac-IB and those after intravenous administration of R-IB or S-IB. Itoh et al. (1997) demonstrated that the enantiomer-enantiomer interaction in plasma protein binding affected the stereoselective pharmacokinetics of IB. Therefore, with intravenous

![Fig. 4. Relative protein levels of ACS1 and APCE in hepatic microsomes of control and AA rats. Results are expressed as the mean ± S.D. (n = 5 or 6). Significant differences between control and AA rats are indicated. ***P < 0.001.](image)

### TABLE 6
Glucuronidation activities for R-IB and S-IB and P450 contents in control and AA rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucuronidation activity</td>
<td>R-IB</td>
<td>S-IB</td>
</tr>
<tr>
<td>(nmol/min/mg protein)</td>
<td>0.23 ± 0.03</td>
<td>0.71 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>0.10 ± 0.01</td>
<td>0.40 ± 0.04</td>
</tr>
<tr>
<td>P450 contents</td>
<td>0.69 ± 0.10</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td>(nmol/mg protein)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$aP < 0.05$ compared with controls.

$bP < 0.05$ compared with its antipode.

### TABLE 7
Plasma levels of total protein and albumin in control and AA rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>6.9 ± 0.1</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.7 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
</tbody>
</table>

$aP < 0.05$ compared with controls.
administration of rac-IB, it is necessary to consider the interactions between R-IB and S-IB in addition to the pharmacokinetics of each enantiomer.

The plasma concentrations of S-IB were significantly higher compared with those of R-IB after intravenous administration of rac-IB (Fig. 2). Similar stereoselective pharmacokinetics was observed for ketoprofen or fenoprofen (Rubin et al., 1985; Jamali and Brocks, 1990), suggesting that the higher CLtot of ketoprofen or fenoprofen (Rubin et al., 1985; Jamali and Brocks, 1990) compared with that of R-IB could be due to the process of chiral inversion in CLtot of R-IB. Similar values for CLMET between R-IB and S-IB were observed in control and AA rats (Table 2). The CLint of S-IB or R-IB could be expressed by the following equations:

\[ \text{CL}_{\text{int}}(S) = \text{CL}_{\text{MET}}(S) = f_u(S) \times \left( \text{CL}_{\text{ox}}(S) + \text{CL}_{\text{glu}}(S) \right) \]  
\[ \text{CL}_{\text{int}}(R) = \text{CL}_{\text{MET}}(R) + \text{CL}_{\text{RS}} = f_u(R) \times \left( \text{CL}_{\text{ox}}(R) + \text{CL}_{\text{glu}}(R) \right) \]

CLox and CLglu indicate the metabolic clearance of unbound IB for hydroxylation and glucuronidation, respectively. The following equation was derived from eq. 12.

\[ \text{CL}_{\text{MET}}(R) = f_u(R) \times \left( \text{CL}_{\text{ox}}(R) + \text{CL}_{\text{glu}}(R) \right) \]

It is possible that the hydroxylation activity for S-IB was dominant compared with that of R-IB because similar values of CLMET were exhibited despite the higher fu and CLglu of S-IB (Tables 6 and 8). Hamman et al. (1997) and Chang et al. (2008) showed the similar extents of stereoselective metabolisms of IB enantiomers by CYP2C. In in vivo study, P450-mediated cleavage is more important for the S-IB (~70%) compared with the R-IB (~30%), because the unidirectional chiral inversion of R-IB to S-IB occurs (Rudy et al., 1991; Davies 1998). Glucuronidation activity for S-IB was 3 to 4 times higher than R-IB (Table 6), although the detailed mechanisms of preferential glucuronidation of S-IB is unclear. Our results concur with the report of el Mouelhi et al. (1987) that S-IB tends to convert to glucuronic acid compared with R-IB.

R-IB was undetectable in plasma after intravenous administration of S-IB (Fig. 3A), whereas S-IB was observed in plasma after intravenous administration of R-IB (Fig. 3B). These results were consistent with those of the studies by Knihinicki et al. (1991), Itoh et al. (1997), and Chen et al. (1991) on the unidirectional chiral inversion in rat. The F1 of IB in control was about 50%, indicating that a half of R-IB underwent chiral inversion to S-IB. This result agreed with those of the studies by Knihinicki et al. (1991), Itoh et al. (1997), and Lee et al. (1984). Interestingly, little difference was observed between the F1 and T1/2inv values of control and those of AA (Table 5). Chiral inversion comprises the following four steps (Knihinicki et al., 1989; Menzel et al., 1994): 1) the formation of R-ibuprofenyl-adenylate from R-IB, 2) the activation process from R-ibuprofenyl-adenylate to R-IB-CoA thioester by long-chain fatty acid ACS, 3) the racemization process of R-IB-CoA thioester by 2-aryl propionyl-CoA epimerase, and 4) the process of release of free IB by hydrolysis enzyme. APCE is present mainly in the liver and kidney (Shieh and Chen, 1993; Reichel et al., 1997). In inflammatory conditions, the activities of metabolic enzymes in the liver reduced as shown in this study (Table 6) and by other groups (Toda et al., 1994; Meunier and Verbeek 1999a). However, in vivo efficiencies of chiral
inversion of IB was not affected by AA. The protein levels of APCE1 but not ACS1 in AA rats significantly decreased compared with control rats (Fig. 4). These results suggested that the efficiencies of epimerization but not the formation of CoA thioester in chiral inversion from R-IB to S-IB could decrease in AA rats, if the protein binding (free fraction) is unchanged in AA rats. However, the decreased protein levels of APCE in AA rats have little effect on the chiral inversion ratio of IB, because the rate-limiting step in the chiral inversion of IB is CoA thioester formation but not epimerization and ibuprofen-CoA formation. (Khininchik et al., 1989; Knudall and Hall, 1990; Knights et al., 1991; Tracy and Hall, 1992). Few reports are available on the chiral inversion of S-IB to R-IB. The CLrac, CLres, and CLmet depend on both the metabolic activities and the protein binding of IB. The plasma unbound fraction of IB in AA rats was 2 to 3 times higher than that in control rats (Table 8). The glucuronidation activity and P450 contents in AA rats significantly decreased (Table 6). The P450 activities in AA rats also decreased, e.g., Cyp3a activities in AA rats reduced to approximately 20% of control (data not shown). As a cause of disagreements between the changes of CL and chiral inversion ratio in AA rats, there is a possibility that the decreased activities of P450, UGT, and enzymes catalyzing chiral inversion in AA rats are counterbalanced by the increased plasma free fraction of IB. Consequently, the ratios of chiral inversion were unchanged between control and AA rats. The effects of alterations in protein binding for IB on IB pharmacokinetics could possibly be larger than those in AA rats in the first. Consequently, the ratios of chiral inversion could be unchanged between control and AA rats.

AA rats exhibit systemic inflammatory disease with changes to bone and cartilage similar to those observed in humans with rheumatoid arthritis (Williams, 1992). The effects of inflammation in arthritic patients on stereoselective pharmacokinetics is very interesting. However, it is unclear whether the human arthritis affects the stereoselective pharmacokinetics of 2-aryl propionic NSAIDs such as IB. A further study of changes of chiral inversion in arthritic patients should be conducted.

In conclusion, remarkable effects of AA on CLrac of S-IB were observed compared with that of R-IB without changes in chiral inversion ratios from R-IB to S-IB after intravenous administration of R-IB or S-IB, although there were slight alterations in the effects of AA on CLrac between stereoisomers after intravenous administration of rac-IB. These changes in the stereoselective pharmacokinetics of IB via decreased activity of P450 and UGT and elevated f_un could affect drug efficiencies.

Authorship Contributions
Participated in research design: Ikuta and Kawase.
Conducted experiments: Ikuta and Kawase.
Performed data analysis: Ikuta and Kawase.
Wrote or contributed to the writing of the manuscript: Kawase and Ikuta.

References


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