Title Page

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

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Running Title: Stereoselective pharmacokinetics of ibuprofen in inflammation

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ABBREVIATIONS: AA, adjuvant-induced arthritis; ACS, acyl-coenzyme A synthetase; 2-APA, 2-arylpropionic acid; APCE, 2-aryl propionyl-coenzyme A epimerase; AUC, area under the plasma concentration-time curve; $CL_{int}$, intrinsic hepatic clearance; $CL_{MET}$, metabolic clearance; $CL_{iRS}$, chiral inversion clearance; $CL_{tot}$, total clearance;
CYP, cytochrome P450; \( f_u \), unbound fractions; HPLC, high-performance liquid chromatography; IB, ibuprofen; IBG, ibuprofen glucuronide; \( i.v. \), intravenous; \( k_{RS} \), inversion rate constants; \( rac \), racemic mixtures; \( T_{1/2\text{inv}} \), half-life for inversion; UGT, UDP-glucuronosyltransferase; \( V_1 \), central compartment; \( V_2 \), peripheral compartment; \( V_{dss} \), distribution volumes at steady state.
Abstract

2-Arylpropionic acid (2-APA) nonsteroidal anti-inflammatory drugs are commonly used in racemic mixtures (rac) for clinical use. 2-APA undergo 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 (CYP) 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 (i.v.) 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 CYP contents of liver microsomes in AA rats were determined. Total clearance (CL\text{tot}) of IB significantly increased in AA rats although the glucuronidation activities for IB, and CYP contents of liver microsomes decreased in AA rats. We presumed that the increased CL\text{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\text{tot} of R-IB but not S-IB significantly increased in AA rats after i.v. administration of rac-IB. These results suggested that AA
could affect drug efficacies following 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 undergo 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; Knihinicki et al., 1989; Chen et al., 1990; Muller 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, 1986; Foster et al., 1988; Jamali et al., 1988; Pedrazzini et al., 1988; Jamali and Brocks, 1990; Brocks 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;
Uno et al., 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 et al., 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; Piquette-Miller and Jamali, 1995), acebutolol (Piquette-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 (CYP) and UDP-glucuronosyltransferase (UGT). Most 2-APA drugs are highly bound to plasma albumin and are mainly eliminated by hepatic metabolism. Meunier and Verbeeck reported the stereoselective glucuronidation of ketoprofen in AA rats (Meunier and Verbeeck, 1999a). 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 ($CL_{int}$) (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 stereoselective pharmacokinetics of $R$-IB and $S$-IB and chiral inversion of IB after intravenous ($i.v.$) 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, USA) 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, USA) and Nippon Shinyaku (Kyoto, Japan), respectively. Rabbit polyclonal anti-acyl-coenzyme A synthetase (ACS)1 antibody (Bioss Antibodies, Woburn, MA, USA), rabbit polyclonal anti-2-aryl propionyl-coenzyme A epimerase (APCE) antibody (Cell Signaling Technology, Danvers, MA, USA), and mouse monoclonal anti-β-actin (Acris 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 mg heat-killed *Mycobacterium butyricum* was subcutaneously injected into the right hind footpad and tail base of rats (Difco Laboratories, Detroit, MI, USA) 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 ID, 1.0 mm 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 *i.v.* 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
Blood samples were collected 1, 5, 10, 20, 30, 45, 60, 90, and 120 min after administration and were centrifuged for 10 min at 3,000 × g. Because of the instability of acyl glucuronides at physiological pH, plasma was transferred to a tube containing 5 μl 17% phosphoric acid. The plasma was immediately frozen on dry ice and stored at −80 °C until analysis.

**Protein binding study**

*In vitro* filtrated unbound IB at a total concentration of 50 μg/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 *i.v.* 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 CYP contents in liver microsomes**

The UGT activities in rat liver microsomes for IB enantiomers were determined.
After incubating 1 mM IB for 10 min in the microsomal suspension in 0.1 M Tris-HCl buffer (pH 7.4) including 1 mg/ml microsome, 10 mM MgCl₂, 0.2% Triton X-100, 2 mM phenyl methyl sulfonyl fluoride, 20 mM 1,4-saccharolactone, and 10 mM UDP-glucuronic acid, the concentrations of IB glucuronide (IBG) formed were stereoselectively determined by HPLC. CYP contents in rat liver microsomes were determined by the method described by Omura and Sato (Omura and Sato, 1964).

Sample preparation for HPLC analysis

Naproxen (50 µl) in methanol (1 µg/ml) was added as an internal standard to mixtures of 100 µl plasma and 400 µl acetic acid (pH 2.5). After the addition of 2 ml ethyl acetate, the mixture was shaken for 10 min, and centrifuged at 2,000 ×g for 5 min. 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-µl residue dissolved in 100 µl of mobile phase was injected into the HPLC system. For bile samples, 50 µl of etodolac in methanol (5 µg/ml) as an internal standard and 100 µl of methanol were added to bile diluted 10 times with purified water. The mixture was vortexed for 30 s and centrifuged at 2,000 ×g for 5 min., and the 20 µl supernatant was directly injected into the HPLC system.
**HPLC conditions**

HPLC analysis was performed using a conventional ODS column (Cosmosil 5C18-AR-α, 4.6 × 250 mm, 5 μm, Nacalai Tesque, Kyoto, Japan) for rac-IB and a chiral column (Chiral OJ-R, 4.6 × 150 mm, 5 μm, Daicel Chemical, Tokyo, Japan) for R-IB, S-IB, R-IBG, and S-IBG using a Shimadzu HPLC system equipped with a UV 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 (AIC) of two-compartment model was smaller than that of one-compartment model. Pharmacokinetic parameters were obtained by the non-linear least square method using
WinNonlin software (Pharsight, Mountain View, CA, USA). Plasma concentration–time profiles of  \( S \)-IB after \( i.v. \) administration of \( S \)-IB were fitted to the following equations:

\[
V_1(S) \frac{dC_1(S)}{dt} = -\left( k_{10(S)} + k_{12(S)} \right) V_1(S) \cdot C_1(S) + k_{21(S)} \cdot X_2(S) \quad (1)
\]

\[
\frac{dX_2(S)}{dt} = k_{12(S)} \cdot X_1(S) - k_{21(S)} \cdot X_2(S) \quad (2)
\]

where \( C_1(S) \) is the plasma concentration of \( S \)-IB in the central compartment; \( X_1(S) \) and \( X_2(S) \) are the amounts of \( S \)-IB in the central and peripheral compartments, respectively; \( V_1(S) \) is the volume of distribution of the central compartment; \( k_{10(S)}, k_{12(S)}, \) and \( k_{21(S)} \) are the first-order rate constants as shown in Fig. 1.

Plasma concentration–time profiles of \( R \)-IB and \( S \)-IB after \( i.v. \) administration of \( R \)-IB were fitted to the following equations:

\[
V_1(R) \frac{dC_1(R)}{dt} = -\left( k_{0(R)} + k_{12(R)} + k_{RS} \right) V_1(R) \cdot C_1(R) + k_{21(R)} \cdot X_2(R) \quad (3)
\]

\[
\frac{dX_2(R)}{dt} = k_{12(R)} \cdot X_1(R) - k_{21(R)} \cdot X_2(R) \quad (4)
\]

\[
V_1(S) \frac{dC_1(S)}{dt} = k_{RS} \cdot X_1(S) - \left( k_{10(S)} + k_{21(S)} \right) V_1(S) \cdot C_1(S) + k_{21(S)} \cdot X_2(S) \quad (5)
\]

\[
\frac{dX_2(S)}{dt} = k_{12(S)} \cdot X_1(S) - k_{21(S)} \cdot X_2(S) \quad (6)
\]

where \( C_1(R) \) is the plasma concentration of \( R \)-IB in the central compartment; \( V_1(R) \) is the volume of distribution of the central compartment; \( k_{10(R)} = k_{0(R)} + k_{RS}, \) and \( k_{0(R)}, k_{RS}, k_{12(R)}, \) and \( k_{21(R)} \) are the first-order rate constants as shown in Fig. 1. For the fitting of
plasma concentration–time profiles of \( S \)-IB after \( i.v. \) administration of \( R \)-IB, parameter values obtained for \( S \)-IB after \( i.v. \) administration of \( S \)-IB were used for \( V_{1(S)}, k_{10(S)}, k_{12(S)}, \) and \( k_{21(S)} \). Therefore, \( V_{1(R)}, k_{10(R)}, k_{0(R)}, k_{RS}, k_{12(R)}, \) and \( k_{21(R)} \) were estimated from the fitting.

\( CL_{tot} \), chiral inversion clearance (\( CL_{RS} \)), and metabolic clearance (\( CL_{MET} \)) were calculated using the following equations:

\[
CL_{tot} = V_1 \times k_{10} 
\tag{7}
\]

\[
CL_{RS} = V_1 \times k_{RS} 
\tag{8}
\]

\( R \)-IB; \( CL_{MET} = CL_{tot} - CL_{RS}, \ S \)-IB; \( CL_{MET} = CL_{tot} \) \( \tag{9} \)

The fraction inverted from \( R \)-IB to \( S \)-IB after \( R \)-IB administration (\( Fi \)) was calculated using three approaches: the area under the plasma concentration-time curve (\( AUC \)) comparison, the deconvolution, and pharmacokinetic modeling methods. The fractional inversion using \( AUC \) (\( Fi_{AUC} \)) was calculated using the principles discussed by Pang and Kwan with the following equation (Pang and Kwan, 1983).

\[
Fi_{AUC} = \frac{AUC_{R \rightarrow S} \cdot Dose_S}{AUC_{S \rightarrow R} \cdot Dose_R} 
\tag{10}
\]

where \( AUC_{R \rightarrow S} \) is the \( AUC_{0-\infty} \) of the metabolically formed \( S \)-IB after \( R \)-IB.
administration and $AUC_{S\rightarrow S}$ is the $AUC_{0\rightarrow\infty}$ of $S$-IB after $S$-IB administration, assuming equal doses of $R$-IB and $S$-IB and no inversion from the $S$-IB to the $R$-IB (Adams et al., 1976; Hutt and Caldwell, 1983).

**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 (SDS-PAGE) was performed using 7.5% e-Pagell (Atto, Tokyo, Japan) and 50 μg microsome per well. Resolved proteins were transferred onto Hybond-P polyvinylidene difluoride membranes (GE Healthcare, Milwaukee, WI, USA). Immunoreactive ACS1, APCE, and β-actin were detected using antibodies, and an ECL Prime Western Blotting Detection system (GE Healthcare).

**Statistical analysis**

The significant difference of the mean values between groups was estimated using analysis of variance followed by the Bonferroni test. A $p$-value of <0.05 was considered statistically significant.
Results

The plasma concentration–time profiles of IB enantiomers following i.v. 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 were 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 was observed; for R-IB, relatively higher total clearance ($CL_{tot}$) 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 ($V_{dss}$) between enantiomers, although the volumes of R-IB tended to be large. The $CL_{tot}$ of each enantiomer was approximately 1.5 times increased in AA rats compared with that of control rats, although the increase in $CL_{tot}$ 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 ($V_1$) and the peripheral compartment ($V_2$) 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 ($CL_{MET}$) between enantiomers in either control or AA rats. However, the $CL_{tot}$ of $R$-IB was significantly higher than that of $S$-IB owing to the inversion of $R$-IB to $S$-IB. The inversion clearance ($CL_{RS}$) and $CL_{MET}$ of $R$-IB increased in AA rats; the $CL_{tot}$, $CL_{RS}$, and $CL_{MET}$ values were approximately 1.5 times higher in AA rats. On the other hand, there were no significant differences in $CL_{tot}$ and $CL_{MET}$ of $S$-IB between control and AA rats. Slight differences in $k_{01(R)}$, $k_{12}$, $k_{21}$, $k_{10}$, and $k_{RS}$ were observed between control and AA rats. The inversion rate constants ($k_{RS}$) for $R$-IB in control and AA rats were 0.044 min$^{-1}$ and 0.052 min$^{-1}$, respectively, which accounted for approximately 70% of $k_{10}$.

To further clarify the effects of AA on the pharmacokinetics of each enantiomer, plasma concentration profiles of the IB enantiomers following the i.v. administration of either $R$-IB or $S$-IB were examined (Fig. 3). $R$-IB was not detected in plasma following $S$-IB administration, indicating that no or negligible inversion from $S$-IB to $R$-IB occurred in rats. $S$-IB was observed in plasma following $R$-IB administration, demonstrating the in vivo inversion of $R$-IB to $S$-IB. Plasma concentrations of $S$-IB increased up to approximately 20 min 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 min onward. The noncompartmental and
compartmental pharmacokinetic parameters of \( R \)-IB and \( S \)-IB after i.v. administration of each enantiomer were estimated (Tables 3 and 4). The \( T_{1/2} \) and \( V_{dss} \) for \( R \)-IB and \( S \)-IB did not change between control and AA rats. The \( CL_{tot} \) of \( R \)-IB and \( S \)-IB in AA rats significantly increased compared with those in control rats. The \( AUCs \) of each enantiomer after administration of the respective enantiomer and of \( S \)-IB after \( R \)-IB administration in AA rats significantly decreased compared with those in control rats. Table 4 demonstrates the tendency for \( V_1 \) and \( V_2 \) to be increased in AA rats following \( rac \)-IB administration. The \( CL_{tot} \) and \( CL_{MET} \) of \( R \)-IB and \( S \)-IB in AA rats significantly increased compared with those in control rats, the results being similar to those after i.v. administration of \( rac \)-IB. The fraction inverted from \( R \)-IB to \( S \)-IB after \( R \)-IB administration (\( F_i \)) and the half-life for inversion (\( T_{1/2inv} \)) in control and AA rats were estimated using \( AUC \) analysis, deconvolution method, and model analysis (Table 5). The \( F_i \) values of \( R \)-IB in both control and AA rats were approximately 50%, indicating that half of \( R \)-IB underwent chiral inversion to \( S \)-IB and that little difference in \( F_i \) values was observed between control and AA rats.

The protein levels of ACS1 and APCE in hepatic microsomes of control and AA rats were determined (Fig. 4). ACS1 and APCE are involved in chiral inversion of IB. The protein levels of ACS1 were unchanged between control and AA rats. The
significant decreases of APCE expression in AA rats were observed.

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 CYP 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 CYP 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). Serum total protein and albumin concentrations in AA rats significantly decreased compared with those in control rats. In particular, the decreases in albumin concentrations in AA rats were marked (approximately 65% of control). Both *in vitro* and *in vivo* plasma unbound fractions ($f_u$) of both enantiomers of IB in AA rats increased approximately 2.5 times compared with those in controls, although $f_u$ of S-IB were significantly greater than those of R-IB in both control and AA rats.
Discussion

In arthritis, CYP activities and plasma 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 CYP activities and plasma protein levels (Walker et al., 1986; Pollock et al., 1989; Piquette-Miller and Jamali, 1992; Piquette-Miller and Jamali, 1995; Meunier and Verbeeck, 1999b). For example, the \( CL_{tot} \) 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 \( CL_{tot} \) 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, \( CL_{tot} \) of IB significantly increased although the glucuronidation activities for IB and CYP contents in liver microsomes decreased to approximately 50% and 70%, respectively, although it is unclear whether AA affects the protein or activity of UGT and CYP isoforms. Probably, the increased \( CL_{tot} \) of IB was caused by the elevated levels of \( f_u \) of IB due to the decreased plasma albumin levels in AA rats (Tables 7 and 8). We previously demonstrated that the changed plasma protein 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 i.v. 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 CYP 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 CYP 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 et al. showed that $CL_{tot}$ of ketoprofen in AA rats did not significantly change (Meunier and Verbeeck, 1999b). 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 (Nagao et al., 2003; Borga and Borga, 1997). After i.v. 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 i.v. administration of rac-IB and those after i.v. administration of R-IB or S-IB. Itoh et al. demonstrated that the enantiomer–enantiomer interaction in plasma protein binding affected the
stereoselective pharmacokinetics of IB (Itoh et al., 1997)). Therefore, with i.v. 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 i.v. administration of rac-IB (Fig. 2). Similar stereoselective pharmacokinetics was observed for ketoprofen or fenoprofen (Jamali and Brocks, 1990; Rubin et al., 1985), suggesting that the higher $CL_{tot}$ of R-IB compared with that of S-IB could be due to the process of chiral inversion in $CL_{tot}$ of R-IB. Similar values for $CL_{MET}$ between R-IB and S-IB were observed in control and AA rats (Table 2). The $CL_{tot}$ of S-IB or R-IB could be expressed by the following equations.

\[
CL_{tot(S)} = CL_{MET(S)} = f_u(s) \times CL_{int(S)} = f_u(s) \times (CL_{ox(S)} + CL_{glu(S)}) \quad (11)
\]

\[
CL_{tot(R)} = CL_{MET(R)} + CL_{RS} = f_u(r) \times CL_{int(R)} = f_u(r) \times (CL_{ox(R)} + CL_{glu(u(R))} + CL_{RS(int)}) \quad (12)
\]

$CL_{ox}$ and $CL_{glu}$ indicate the metabolic clearance of unbound IB for hydroxylation and glucuronidation, respectively. The following equation was derived from equation (12).

\[
CL_{MET(R)} = f_u(r) \times (CL_{ox(R)} + CL_{glu(R)}) \quad (13)
\]

It is possible that the hydroxylation activity for S-IB was dominant compared with that
of R-IB because similar values of $CL_{MET}$ were exhibited despite the higher $f_u$ and $CL_{glu}$ of S-IB (Tables 6 and 8). Hamman et al. and Chang et al. showed the similar extents of stereoselective metabolisms of IB enantiomers by CYP2C (Hamman et al., 1997; Chang et al., 2008). In in vivo study, CYP-mediated clearance 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 Mouelhi’s report that S-IB is tend to convert to glucuronide compared with R-IB (et Mouelhi et al., 1987).

R-IB was undetectable in plasma after i.v. administration of S-IB (Fig. 3A), whereas S-IB was observed in plasma after i.v. administration of R-IB (Fig. 3B). These results were consistent with those of the studies by Knihinicki et al. (Knihinicki et al., 1991), Itoh et al. (Itoh et al., 1997), and Chen et al. (Chen et al., 1991) on the unidirectional chiral inversion in rat. The $Fi$ 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. (Knihinicki et al., 1991), Itoh et al. (Itoh et al., 1997), and Lee et al (Lee et al., 1984). Interestingly, little difference was observed between the
Fi and $T_{1/2\text{inv}}$ 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 Verbeeck 1999a). However, in vivo efficiencies of chiral inversion of IB were 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 ibuprofenyl-CoA formation (Knihinicki et al., 1989; Knadler et al., 1990; Knights et al., 1991; Tracy et al., 1992). Few reports are
available on the chiral inversion of $S$-IB to $R$-IB. The $CL_{408}$, $CL_{RS}$, and $CL_{MET}$ 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 CYP contents in AA rats significantly decreased (Table 6). The CYP 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 CYP, 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 be possibly larger than those in APCE activities in AA rats. 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 et al., 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 $CL_{tot}$ of $S$-IB were observed compared with that of $R$-IB without changes in chiral inversion ratios from $R$-IB to $S$-IB after i.v. administration of $R$-IB or $S$-IB, although there were slight alterations in the effects of AA on $CL_{tot}$ between stereoisomers after i.v. administration of $rac$-IB. These changes in the stereoselective pharmacokinetics of IB via decreased activity of CYP and UGT and elevated $f_u$ could affect drug efficiencies.
Acknowledgments

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Authorship Contributions

*Participated in research design:* Masahiro Iwaki

*Conducted experiments:* Hiroyuki Ikuta and Atsushi Kawase

*Performed data analysis:* Hiroyuki Ikuta and Atsushi Kawase

*Wrote or contributed to the writing of the manuscript:* Atsushi Kawase and Masahiro Iwaki
References


DMD #73239

Pharmacokinet 28: 100-114.


Uno S, Kawase A, Tsuji A, Tanino T, and Iwaki M (2007) Decreased intestinal CYP3A


Footnotes

This work was supported by the “Antiaging” Project for Private Universities, with a matching fund subsidy from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT); the MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2014-2018 [S1411037].
Figure legends

Fig. 1. A pharmacokinetic model with unidirectional inversion from R-IB to S-IB.

Fig. 2. Plasma concentration–time profiles of IB enantiomers after i.v. administration of rac-IB (20 mg/kg) in control and AA rats. Δ, R-IB; ○, S-IB in control rat; ▲, R-IB; ●, S-IB in AA rats. Solid lines represent the fitting curves using the pharmacokinetic model shown in Fig. 1. Results are expressed as the mean ± SD (n = 3).

Fig. 3. Plasma concentration–time profiles of IB enantiomers after i.v. administration of S-IB (10 mg/kg) (A) or R-IB (10 mg/kg) (B) in control and AA rats. Δ, R-IB; ○, S-IB in control rat; ▲, R-IB; ●, S-IB in AA rats. Solid lines represent the fitting curves using the pharmacokinetic model shown in Fig. 1. Results are expressed as the mean ± SD (n = 3).

Fig. 4. Relative protein levels of ACS1 and APCE in hepatic microsomes of control and AA rats. Results are expressed as the mean ± SD (n = 5–6). Significant differences between control and AA rats are indicated (**p < 0.001).

Fig. 5. Effects of AA induction on CLRS (ml/min/kg) and CLMET (ml/min/kg) of IB.
Tables

Table 1. Noncompartmental pharmacokinetic parameters of each enantiomer after i.v. administration of rac-IB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R-IB</td>
<td>S-IB</td>
</tr>
<tr>
<td>$T_{1/2}$ (min)</td>
<td>35.7 ± 6.8</td>
<td>89.5 ± 37.1</td>
</tr>
<tr>
<td>$CL_{tot}$ (ml/min/kg)</td>
<td>3.70 ± 0.14</td>
<td>0.80 ± 0.27</td>
</tr>
<tr>
<td>$MRT$ (min)</td>
<td>32.7 ± 3.9</td>
<td>127 ± 51.9</td>
</tr>
<tr>
<td>$V_d$ (ml/kg)</td>
<td>120 ± 18</td>
<td>89 ± 10</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ ($\mu$g ∙ min/ml)</td>
<td>2720 ± 103</td>
<td>14031 ± 4496</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SD (n = 3). a) $p < 0.05$ compared with controls. b) $p < 0.05$ compared with its antipode.
Table 2. Compartmental pharmacokinetic parameters of each enantiomer after *i.v.* administration of *rac*-IB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>R</em>-IB</td>
<td><em>S</em>-IB</td>
</tr>
<tr>
<td>$V_1$ (ml/kg)</td>
<td>63.1 ± 4.4</td>
<td>67.3 ± 9.9</td>
</tr>
<tr>
<td>$V_2$ (ml/kg)</td>
<td>56.4 ± 24.6</td>
<td>56.0 ± 23.2</td>
</tr>
<tr>
<td>$CL_{tot}$ (ml/min/kg)</td>
<td>4.01 ± 0.40</td>
<td>1.46 ± 0.40*</td>
</tr>
<tr>
<td>$CL_{RS}$ (ml/min/kg)</td>
<td>2.75 ± 0.39</td>
<td>2.19 ± 0.55*</td>
</tr>
<tr>
<td>$CL_{MET}$ (ml/min/kg)</td>
<td>1.26 ± 0.09</td>
<td>1.46 ± 0.40</td>
</tr>
<tr>
<td>$k_{01}$ (min$^{-1}$)</td>
<td>0.020 ± 0.001</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>
</tr>
<tr>
<td>$k_{21}$ (min$^{-1}$)</td>
<td>0.074 ± 0.024</td>
<td>0.062 ± 0.020</td>
</tr>
<tr>
<td>$k_{03}$ (min$^{-1}$)</td>
<td>0.064 ± 0.007</td>
<td>0.021 ± 0.003*</td>
</tr>
<tr>
<td>$k_{30}$ (min$^{-1}$)</td>
<td>0.044 ± 0.007</td>
<td>0.052 ± 0.022</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SD (n = 3). a) $p < 0.05$ compared with controls. b) $p < 0.05$ compared with its antipode.
Table 3. Noncompartmental pharmacokinetic parameters of each enantiomer after *i.v.* administration of *R*-IB or *S*-IB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>R</em>-IB after</td>
<td><em>S</em>-IB after</td>
</tr>
<tr>
<td><em>T</em>&lt;sub&gt;1/2&lt;/sub&gt; (min)</td>
<td>33.9 ± 4.5</td>
<td>77.8 ± 24.4</td>
</tr>
<tr>
<td><em>CL</em>&lt;sub&gt;tot&lt;/sub&gt; (ml/kg)</td>
<td>4.20 ± 0.23</td>
<td>—</td>
</tr>
<tr>
<td><em>MRT</em> (min)</td>
<td>32.8 ± 5.4</td>
<td>—</td>
</tr>
<tr>
<td><em>Vd&lt;sub&gt;ss&lt;/sub&gt;</em> (ml/kg)</td>
<td>137 ± 18</td>
<td>—</td>
</tr>
<tr>
<td><em>AUC</em> (mg∙min/ml)</td>
<td>2386 ± 129</td>
<td>3549 ± 474</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SD (n = 3). a) *p* < 0.05 compared with controls. b) *p* < 0.05 compared with its antipode.
Table 4. Compartmental pharmacokinetic parameters of each enantiomer after *i.v.* administration of *R*-IB or *S*-IB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>AA</th>
</tr>
</thead>
</table>
|                    | *R*-IB                       | *S*-IB                | *R*-IB   | *S*-IB
| $V_1$ (ml/kg)      | 90.1 ± 20.0                  | 67.2 ± 9.8             | 113 ± 38 | 99.3 ± 18.3 |
| $V_2$ (ml/kg)      | 78.5 ± 29.2                  | 93.4 ± 72.2            | 125 ± 52 | 112 ± 69   |
| $CL_{tot}$ (ml/min/kg) | 4.28 ± 0.19                 | 1.26 ± 0.12 $^{b)}$   | 6.87 ± 1.05 $^{a)}$ | 2.83 ± 0.92 $^{a), b)}$ |
| $CL_{RS}$ (ml/min/kg) | 2.21 ± 0.53                 | —                      | 3.81 ± 0.42 $^{a)}$ | —          |
| $CL_{MET}$ (ml/min/kg) | 2.07 ± 0.32                 | 1.26 ± 0.12 $^{b)}$   | 3.05 ± 0.42 $^{a)}$ | 2.83 ± 0.92 $^{a)}$ |
| $k_{0(R)}$ (min$^{-1}$) | 0.023 ± 0.003               | —                      | 0.029 ± 0.009 | —          |
| $k_{12}$ (min$^{-1}$) | 0.026 ± 0.018               | 0.056 ± 0.030          | 0.029 ± 0.010 | 0.058 ± 0.020 |
| $k_{21}$ (min$^{-1}$) | 0.031 ± 0.018               | 0.046 ± 0.020          | 0.033 ± 0.013 | 0.050 ± 0.017 |
| $k_{00}$ (min$^{-1}$) | 0.048 ± 0.008               | 0.019 ± 0.004 $^{b)}$ | 0.060 ± 0.008 | 0.028 ± 0.006 $^{b)}$ |
| $k_{RS}$ (min$^{-1}$) | 0.026 ± 0.011               | —                      | 0.032 ± 0.003 | —          |

Results are expressed as the mean ± SD (n = 3). $a) p < 0.05$ compared with controls. $b) p < 0.05$ compared with its antipode.
Table 5. $F_i$ and $T_{1/2\text{inv}}$ of inversion calculated by $AUC$ analysis, deconvolution method, and model analysis in control and AA rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>AA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC analysis</td>
<td>Deconvolution method</td>
<td>Model analysis</td>
<td>AUC analysis</td>
</tr>
<tr>
<td>$F_i$</td>
<td>$(F_{iAUC})_a$</td>
<td>$(F_{iDECON})_b$</td>
<td>$(F_{iCOMP})_c$</td>
<td>$0.47 \pm 0.07$</td>
</tr>
<tr>
<td>$T_{1/2\text{inv}}$ (min)</td>
<td>$-\quad$</td>
<td>$15.9 \pm 4.4$</td>
<td>$29.7 \pm 10.5$</td>
<td>$-\quad$</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SD (n = 3). a) $F_{iAUC} = \frac{AUC_{R \to S}}{AUC_{S \to R}}$. b) Calculated using the deconvolution method. c) $F_{iCOMP} = \frac{k_{RS}}{k_0(R)} + k_{RS}$. 


Table 6. Glucuronidation activities for \( R \)-IB and \( S \)-IB and CYP contents in control and AA rats

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

Results are expressed as the mean ± SD (n = 3). \(^a\) \( p < 0.05 \) compared with controls. \(^b\) \( p < 0.05 \) 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>
<th>a) p &lt; 0.05 compared with controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>6.9 ± 0.1</td>
<td>5.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>(g/dl)</td>
<td></td>
<td>a)</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.7 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± SD (n = 3).
Table 8. *In vitro* and *in vivo* plasma unbound fractions ($f_u$) (%) of IB in control and AA rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>R</em>-IB</td>
<td><em>S</em>-IB</td>
</tr>
<tr>
<td><strong>In vitro</strong></td>
<td>0.72 ± 0.24</td>
<td>1.38 ± 0.34</td>
</tr>
<tr>
<td><strong>In vivo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post dosing (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.21 ± 0.32</td>
<td>2.96 ± 0.60</td>
</tr>
<tr>
<td>30</td>
<td>2.16 ± 0.29</td>
<td>3.59 ± 0.53</td>
</tr>
<tr>
<td>Average&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.19</td>
<td>3.27</td>
</tr>
</tbody>
</table>

*In vitro;* Rac-IB (at a total concentration of 50 μg/ml). *In vivo;* dose, rac-IB 20 mg/kg.

Results are expressed as the mean ± SD (n = 3). a) Average values between 10 and 30.

b) $p < 0.05$ compared with controls. c) $p < 0.05$ compared with its antipode.
Fig. 1

\[ k_{10(R)} = k_{0(R)} + k_{RS} \]
Fig. 2

[Graph showing plasma concentration (µg/mL) over time (min).]

Plasma concentration (µg/mL) vs Time (min)
Fig. 4

**ACS1**

- Control: 1.0
- AA: 0.9

**APCE**

- Control: 1.0
- AA: 0.7

**Western Blot Analysis**

- ACS1
  - Control: Intense band
  - AA: Less intense band

- β-actin
  - Control: Strong band
  - AA: Strong band

- APCE
  - Control: Weak band
  - AA: Weak band

- β-actin
  - Control: Strong band
  - AA: Strong band
Fig. 5

Control

\[
\begin{align*}
R-IB & \quad CL_{MET} = 2.07 \pm 0.32 \\
S-IB & \quad CL_{MET} = 1.26 \pm 0.12 \\
\end{align*}
\]

AA

\[
\begin{align*}
R-IB & \quad CL_{MET} = 3.05 \pm 0.42 \\
S-IB & \quad CL_{MET} = 2.83 \pm 0.92 \\
\end{align*}
\]