Pharmacokinetic Study of the Hepatobiliary Transport of Indomethacin

Hirokazu Kouzuki,[1] Hiroshi Suzuki,[1,2] and Yuichi Sugiyama[1,2,3]

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Purpose. The biliary excreted amount of indomethacin and its glucuronide is related to the intestinal toxicity of this drug. In the present study, we investigated the hepatobiliary transport of indomethacin.

Methods. The uptake of indomethacin into primary cultured rat hepatocytes and COS-7 cells transfected with cDNA encoding sodium taurocholate co-transporting polypeptide or organic anion transporting polypeptide 1 was examined. Moreover, we compared the biliary excretion of indomethacin and its glucuronide between Sprague-Dawley (SD) rats and Eisai hyperbilirubinemic rats (EHBR) whose canalicular multispecific organic anion transporter/multidrug resistance associated protein 2 (cMOAT/MRP2) function is hereditarily defective.

Results. The uptake of indomethacin into rat hepatocytes was mediated by Na^+-dependent and independent active transport systems. Neither transfectant stimulated the uptake of indomethacin. After intravenous infusion of indomethacin to SD rats, the biliary excretion of indomethacin glucuronide exceeded that of indomethacin. The indomethacin transport clearance across the bile canalicular membrane was comparable between SD rats and EHBR, whereas the corresponding value for indomethacin glucuronide in EHBR was approximately 50% that in SD rats.

Conclusions. These results indicate that another transporter(s) is involved in the hepatic uptake of indomethacin and the canalicular transport of indomethacin glucuronide is mediated by cMOAT/MRP2 whereas that of indomethacin is not mediated by cMOAT/MRP2.

KEY WORDS: indomethacin; hepatic uptake; Ntcp; oatp; biliary excretion; cMOAT/MRP2.

INTRODUCTION

Indomethacin, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid, is a non-steroid anti-inflammatory agent with antipyretic and mild analgesic actions, which has been widely used in the treatment of many arthritic disorders (1,2). Its clinical use, however, is limited due to severe side effects, including intestinal lesions (3). It has been established that the amount of indomethacin and its glucuronide excreted into the bile is closely related to the intestinal toxicity of this anti-inflammatory drug. This has been proposed by Duggan et al. (4), who focused on the interspecies difference in the toxicity of indomethacin. They found a clear correlation between the minimum toxic dose and the amount of indomethacin and its glucuronide excreted in bile among rats, guinea pigs, rabbits, dogs and monkeys (4). The mechanism for the hepatobiliary excretion of indomethacin, however, has remained unknown.

Concerning the hepatic uptake of organic anions, it has been demonstrated that sodium taurocholate (TC) co-transporting polypeptide (Ntcp) and organic anion transporting polypeptide 1 (oatp1) are responsible for the Na^+-dependent uptake of bile acids and Na^+-independent uptake of organic anions, respectively (5). Moreover, the number of membrane proteins cloned as transporters responsible for the hepatic uptake of organic anions has increased in recent years (6–10). The transport properties of these cloned cDNA products have been clarified by examining the substrate uptake into *Xenopus laevis* oocytes injected with cRNA and/or into mammalian cells transfected with cDNA (5–11).

After being taken up by hepatocytes *via* these transporters, organic anions are excreted into the bile with or without metabolic conversion. Using isolated bile canalicular membrane vesicles, it has been demonstrated that several organic anions are transported *via* an ATP-dependent transporter referred to as the canalicular multispecific organic anion transporter/multidrug resistance associated protein 2 (cMOAT/MRP2) (5,12,13). By comparing the transport properties in normal rats and mutants whose cMOAT/MRP2 function is hereditarily defective (such as Eisai hyperbilirubinemic rats (EHBR; 14)), the substrate specificity of cMOAT/MRP2 has been clarified (13,15,16). It has been demonstrated that cMOAT/MRP2 accepts conjugated metabolites of xenobiotics, along with organic anions without metabolic conversion (13,15,16).

In the present study, we investigated the hepatic uptake and biliary excretion mechanisms of indomethacin. We examined the uptake of indomethacin into primary cultured rat hepatocytes and Ntcp- or oatp1 transfected COS-7 cells. Moreover, we compared the biliary excretion of indomethacin and its glucuronide between normal Sprague-Dawley (SD) rats and EHBR.

MATERIALS AND METHODS

Materials

COS-7 cells were purchased from American Type Culture Collection (Rockville, MD). [^{14}C]Indomethacin (0.83 GBq/mmol), [^{3}H]TC (128.4GBq/mmol) and [^{3}H]estradiol 17β-D-glucuronide ($E_2$17βG; 1813 GBq/mmol) were purchased from New England Nuclear (Boston, MA). TC and indomethacin were purchased from Wako Pure chemical Industries (Osaka, Japan). $E_2$17βG was purchased from Sigma Chemical (St.

ABBREVIATIONS: TC, taurocholate; Ntcp, sodium taurocholate co-transporting polypeptide; oatp1, organic anion transporting polypeptide 1; cMOAT/MRP2, canalicular multispecific organic anion transporter/multidrug resistance associated protein 2; EHBR, Eisai hyperbilirubinemic rats; SD rats, Sprague-Dawley rats; $E_2$17βG, estradiol 17β-D-glucuronide; DBSP, dibromosulfophthalein; WE medium, Williams' medium E; DMEM, Dulbecco's modified Eagle's medium; FCCP, carbonyl cyanide-*p*-(trifluoromethoxy)-phenylhydrazone; V_0, initial uptake velocity; K_m, Michaelis constant; V_{max}, maximum transport velocity; P_{dif}, nonspecific uptake clearance; CL_{plasma}, total body clearance; $CL_{bile,\ plasma}$ and $CL_{bile,\ liver}$, biliary excretion clearances defined for plasma and liver concentrations; oat1, organic anion transporter.

[1] Graduate School of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

[2] Japan Science and Technology Corporation, Hongo, Bunkyo-Ku, Tokyo 113-0033, Japan.

[3] To whom correspondence should be addressed. (e-mail: sugiyama@seizai.f.u-tokyo.ac.jp)

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Louis, MO). Dibromosulfophthalein (DBSP) was obtained from Societe d'Etudes et de Recherches Biologiques (Paris, France). 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor (pravastatin) was kindly donated by Sankyo Co., Ltd. (Tokyo, Japan). All other chemicals were commercially available and of reagent grade.

Male SD rats and EHBR were purchased from Japan Laboratory Animal Inc. (Tokyo, Japan) and Eisai Laboratories (Gifu, Japan), respectively.

Primary Cultured Rat Hepatocytes

The procedure for the preparation of primary cultured rat hepatocytes has been described previously (17,18). Briefly, rat hepatocytes were isolated from male SD rats of 200–250 g body weight after perfusion of the liver with collagenase. Cell viability was routinely checked by the trypan blue [0.4% (wt/vol)] exclusion test. After preparation, freshly isolated cells were suspended in Williams' medium E (WE medium). Approximately 5×10^5 cells were placed on collagen-coated 22-mm dishes and cultured for 4 hr.

Transient Expression of Ntcp and oatp1 cDNA in COS-7 Cells

The pCAGGS plasmids containing Ntcp and oatp1 cDNAs were used in the present study. The structure of the plasmid construct has been described previously (17,18).

The procedure for the transfection of plasmids into COS-7 cells has been described previously (17,18). Briefly, COS-7 cells were cultured in 150-mm dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum. At 30% confluence, cells were exposed to serum-free DMEM containing plasmid (1 μg/ml) and Lipofectamine (1 μg/ml, BRL, Gaithersburg, MD). At 8 hr after transfection, the plasmid-Lipofectamine solution was removed and the cells were cultured overnight in a medium consisting of DMEM supplemented with 5% fetal bovine serum. The transfected cells were treated with trypsin and approximately 1.6×10^5 cells were seeded onto 22-mm dishes and cultured overnight. An uptake study was performed at 48 hr after transfection.

Uptake Study

Uptake of [^{14}C]indomethacin (1 and 10 μM for hepatocytes and 10 μM for Ntcp-or oatp1-transfected COS-7 cells), [^{3}H]TC (1 μM) and [^{3}H]$E_2$17βG (1 μM) was examined by the method described previously (17,18). Briefly, after washing the cultured cells 3 times with Krebs-Henseleit buffer or choline buffer, the cells were preincubated at 37°C for 5 min in the respective buffer. The experiments were initiated by adding the radiolabeled ligands to the medium. The Krebs-Henseleit buffer consisted of 142 mM NaCl, 23.8 mM Na_2CO_3, 4.83 mM KCl, 0.96 mM KH_2PO_4, 1.20 mM $MgSO_4$, 12.5 mM HEPES, 5 mM glucose and 1.53 mM $CaCl_2$ adjusted to pH 7.3. The composition of the choline buffer was the same as that of the Krebs-Henseleit buffer except that NaCl and $NaHCO_3$ were replaced with isotonic choline chloride and choline bicarbonate, respectively. For inhibition studies, the cultured cells were pre-incubated with a metabolic inhibitor [2 μM carbonyl cyanide-*p*-(trifluoromethoxy)-phenylhydrazone (FCCP)] at 37°C for 5 min before adding [^{14}C]indomethacin. To determine the inhibitory effect, bile acid (TC), non-bile organic anions (pravastatin, DBSP and $E_2$17βG) or unlabeled indomethacin was added to the cultured cells simultaneously with [^{14}C]indomethacin. At designated times, the reaction was terminated by adding ice-cold Krebs-Henseleit buffer. Just prior to the designated times, 50 μl medium was transferred to scintillation vials. Then, the cells were washed 3 times with 2 ml ice-cold Krebs-Henseleit buffer and solubilized in 500 μl 1 N NaOH. After adding 500 μl distilled water, 800 μl aliquots were transferred to scintillation vials. The radioactivity associated with the cells and medium was determined in a liquid scintillation counter (LS 6000SE, Beckman Instruments, Inc., Fullerton, CA) after adding 8 ml scintillation fluid (Hionic flour, Packard, Instrument Co., Downers Grove, IL) to the scintillation vials. The remaining 100 μl aliquots of cell lysate were used to determine protein concentrations by the method of Lowry et al (19) with bovine serum albumin as a standard. Ligand uptake is given as the cell-to-medium concentration ratio, determined as the amount of ligand associated with the cells divided by the medium concentration. The Na^+-dependent uptake was calculated by subtracting the Na^+-independent uptake (measured in choline buffer) from the total uptake (measured in Krebs-Henseleit buffer). Ntcp- or oatp1-mediated uptake was calculated by subtracting the uptake into COS-7 cells transfected with pCAGGS vector (measured in Krebs-Henseleit buffer) from that into COS-7 cells transfected with pCAGGS containing Ntcp or oatp1 (measured in Krebs-Henseleit buffer).

The kinetic parameters for indomethacin uptake were estimated from the following equation;

$$V_0 = (V_{max} \times S)/(K_m + S) + P_{dif} \times S$$

where V_0 is the initial uptake velocity of ligand (nmol/min/mg protein), S is the concentration of ligand in the medium (μM), K_m is the Michaelis constant (μM), V_{max} is the maximum uptake rate (nmol/min/mg protein) and P_{dif} is the nonspecific uptake clearance (μl/min/mg protein). The uptake data were fitted to this equation by a nonlinear least-squares method using a MULTI program (20) to obtain estimates of the kinetic parameters. The input data were weighted as the reciprocals of the squares of the observed values.

In Vivo Study

SD rats of 302–368 g body weight and EHBR of 288–310 g body weight (n = 5) were lightly anesthetized with diethyl ether, and the femoral artery and vein were cannulated with polyethylene tubing (PE-50) for blood sampling and ligand administration, respectively. The common bile duct was also cannulated with PE-10 to collect bile specimens. [^{14}C]Indomethacin in saline was infused through the femoral vein cannula at a rate for 0.3 mg/min/kg for 2 hr after intravenous bolus injection (0.1 mg/kg). Blood and bile specimens were obtained at specified times. Plasma was prepared by the centrifugation (10,000 × g for 1 min) of blood samples. Aliquots of plasma (50 μl) and bile (50 μl) were transferred to scintillation vials. At the end of the infusion, the liver was excised and rinsed with saline. Approximately 200 mg liver was transferred to scintillation vials and solubilized in 2 ml Soluen 350 (Packard, Instrument Co., Downers Grove, IL). The radioactivity associated with the plasma, bile and liver was determined in a liquid

scintillation counter (LS 6000SE) after adding 8 ml scintillation fluid (Hionic flour) to the scintillation vials.

The amount of [^{14}C]Indomethacin and its glucuronide associated with the biological specimens was determined as follows; a 5-fold volume of ethanol was added to plasma, bile and liver, and the liver was homogenized. After centrifugation of the mixture (10,000 × g for 3 min), the supernatant was analyzed by silica-gel thin-layer chromatography in chloroform: acetic acid (95:5 (21)). The amount of [^{14}C]indomethacin and its glucuronide was quantified using a Bio-Image Analyzer (Bas 2000, Fuji Film, Tokyo, Japan).

Total body clearance (CL_{plasma}) was calculated by dividing the infusion rate of indomethacin by the arterial plasma concentration of indomethacin. Biliary excretion clearances defined for plasma ($CL_{bile,\ plasma}$) and liver ($CL_{bile,\ liver}$) concentrations were calculated by dividing the biliary excretion rate of indomethacin and its glucuronide by the arterial plasma and liver concentration of indomethacin and its glucuronide, respectively.

A paired t-test was used to determine significant differences between the estimated parameters of SD rats and that of EHBR.

RESULTS

In Vitro Studies

The uptake of indomethacin by primary cultured rat hepatocytes was examined. Indomethacin (1 μM) was taken up into the cultured hepatocytes in a time-dependent manner (Fig. 1). Replacement of Na^+ by choline significantly reduced the uptake of indomethacin (Fig. 1). Both Na^+-dependent and independent uptake exhibited a marked temperature dependence and also decreased in the presence of a metabolic inhibitor (2 μM FCCP) (Fig. 1). Kinetic analysis of the uptake of indomethacin by cultured hepatocytes gave a K_m of 11.5 +/− 6.3 μM, a V_{max} of 1.07 +/− 0.35 nmol/min/mg protein and a P_{dif} of 2.13 +/− 1.11 μl/min/mg protein in the presence of Na^+ (Fig. 2).

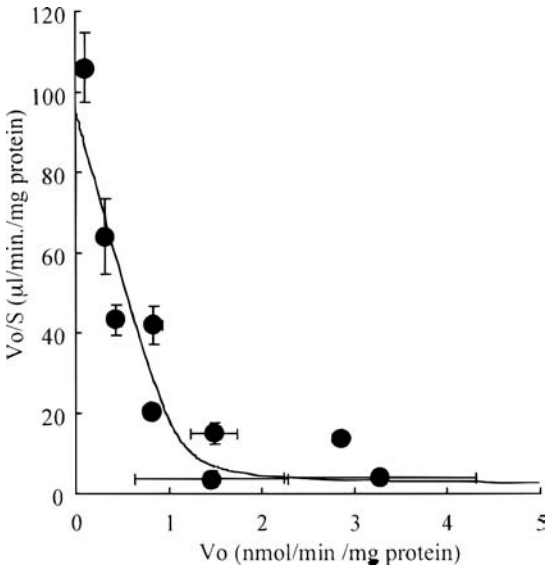

Fig. 2. Eadie-Hofstee plot for indomethacin uptake. Uptake of indomethacin by primary cultured rat hepatocytes was measured at concentrations of 1, 2, 5, 10, 20, 40, 100, 200, 400 and 800 μM in Krebs-Henseleit buffer. Each symbol and vertical bar represents the mean ± S.E. of 3 determinations.

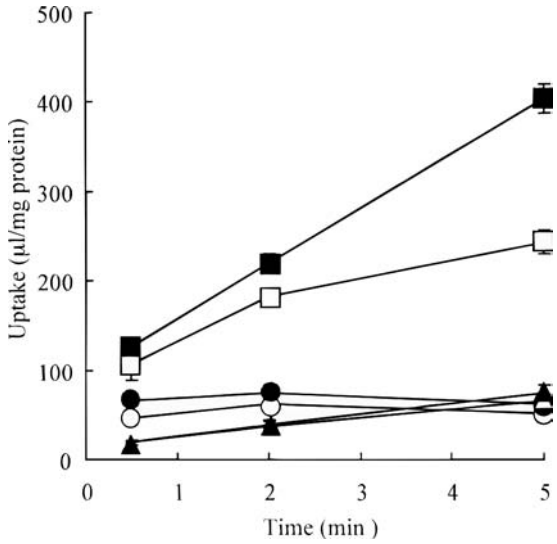

Fig. 1. Time profiles for the uptake of [^{14}C]indomethacin. Uptake of [^{14}C]indomethacin (1 μM) by primary cultured rat hepatocytes was examined under several conditions. Open and closed symbols represent the uptake from choline-buffer and Krebs-Henseleit buffer, respectively. The uptake of [^{14}C]indomethacin was examined at 37°C (squares) and 0°C (triangles). The uptake was also studied in the presence of FCCP (2 μM) (circles). Each symbol and vertical bar represents the mean ± S.E. of 3 determinations.

The inhibitory effects of bile acid (TC) and non-bile acid organic anions on the uptake of indomethacin by primary cultured rat hepatocytes in the presence of Na^+ were determined. The uptake of indomethacin (1 μM) was inhibited by TC and non-bile acid organic anions (pravastatin, DBSP and $E_2$17βG) in a concentration-dependent manner (Fig. 3). To reduce the uptake of indomethacin to approximately 50% of the control,

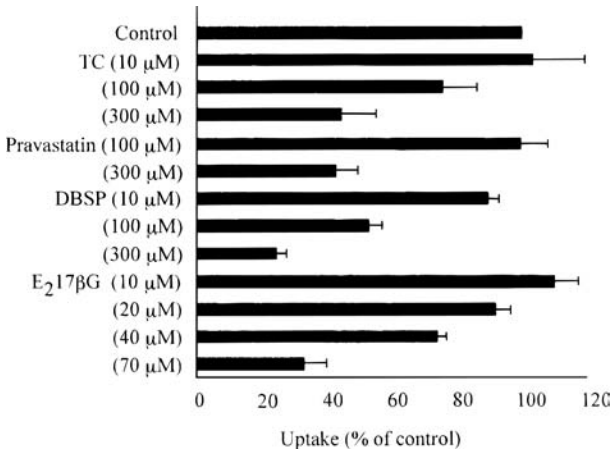

Fig. 3. Effect of organic anions on the uptake of [^{14}C]indomethacin. Uptake of [^{14}C]indomethacin (1 μM) by primary cultured rat hepatocytes was measured in Krebs-Henseleit buffer in the presence of TC, pravastatin, DBSP and $E_2$17βG. The concentrations of inhibitors are indicated in parentheses. Each bar represents the mean ± S.E. of 3 determinations.

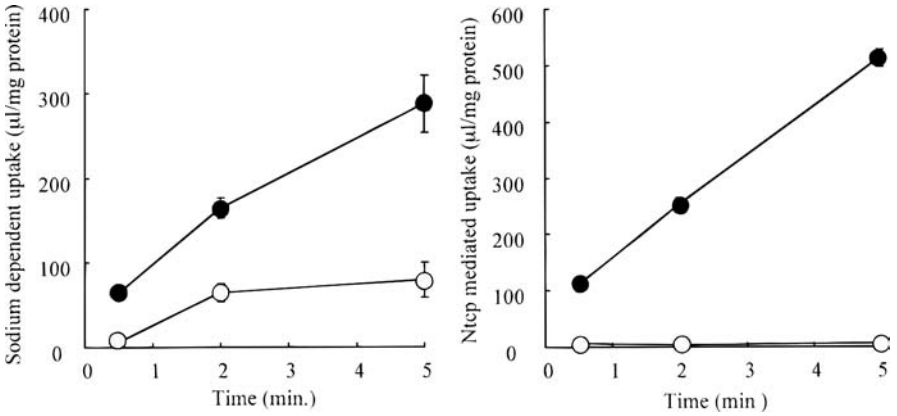

Fig. 4. Time profiles for the uptake of TC and indomethacin. The Na^+-dependent uptake of [^{14}C]indomethacin (10 μM) and [^{3}H]taurocholate (1 μM) by primary cultured rat hepatocytes (left panel) and Ntcp-transfected COS-7 cells (right panel) was examined. Ntcp-mediated uptake represents the difference in the uptake between Ntcp- and control vector transfected cells. Open and closed circles represent the uptake of indomethacin and TC, respectively. Each symbol and vertical bar represents the mean ± S.E. of 3 determinations for primary cultured rat hepatocytes and the corresponding values of 9 determinations in 3 different preparations.

300 μM TC and pravastatin, 100 μM DBSP and 40–70 μM $E_2$17βG were required (Fig. 3).

We also examined Na^+-dependent and Na^+-independent uptake of indomethacin into COS-7 cells transiently expressing Ntcp and oatp1, compared with those into vector-transfected COS-7 cells. Transfection of Ntcp or oatp1 cDNA did not significantly affect the uptake of indomethacin (10 μM) by COS-7 cells (Figs. 4 and 5). In contrast, the uptake of TC or $E_2$17βG into COS-7 cells, typical substrates for Ntcp and oatp1, respectively, was stimulated by transfection of Ntcp or oatp1 cDNA (Figs. 4 and 5).

In Vivo Study

The plasma concentration-time profiles of indomethacin and its glucuronide after intravenous injection followed by constant-rate intravenous infusion of indomethacin to SD rats and EHBR are shown in Fig. 6. No significant differences were observed in the plasma concentration of indomethacin and its glucuronide between SD rats and EHBR (Fig. 6 and Table 1). The biliary excretion rate of indomethacin and its glucuronide are shown in Fig. 7. The biliary excretion rate of indomethacin glucuronide exceeded that of the parent drug in both SD rats and EHBR (Fig. 7). The biliary excretion rate of indomethacin was similar in the two rat strains (Fig. 7) and, as a consequence, the $CL_{bile,\ plasma}$ and $CL_{bile,\ liver}$ for indomethacin were not significantly different between the two rat strains (Table 1). In contrast, the biliary excretion of indomethacin glucuronide was significantly lower in EHBR, compared with SD rats and the $CL_{bile,\ liver}$, for indomethacin glucuronide in EHBR was significantly lower than that of SD rats (Table 1).

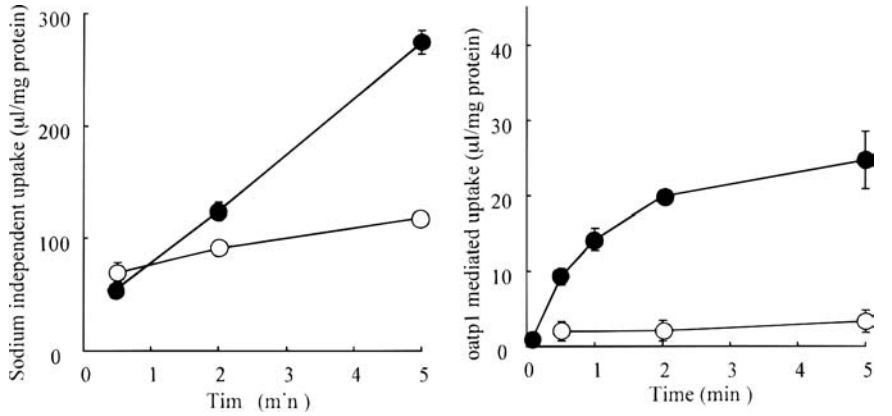

Fig. 5. Time profiles for the uptake of $E_2$17βG and indomethacin. The Na^+-independent uptake of [^{14}C]indomethacin (10 μM) and [^{3}H]$E_2$17βG (1 μM) by primary cultured rat hepatocytes (left panel) and oatp1-transfected COS-7 cells (right panel) was examined. Oatp1-mediated uptake represents the difference in the uptake between oatp1 and control vector transfected cells. Open and closed circles represent the uptake of indomethacin and $E_2$17βG, respectively. Each symbol and vertical bar represents the mean ± S.E. of 3 determinations for primary cultured rat hepatocytes and corresponding values of 6 determinations in 2 different preparations.

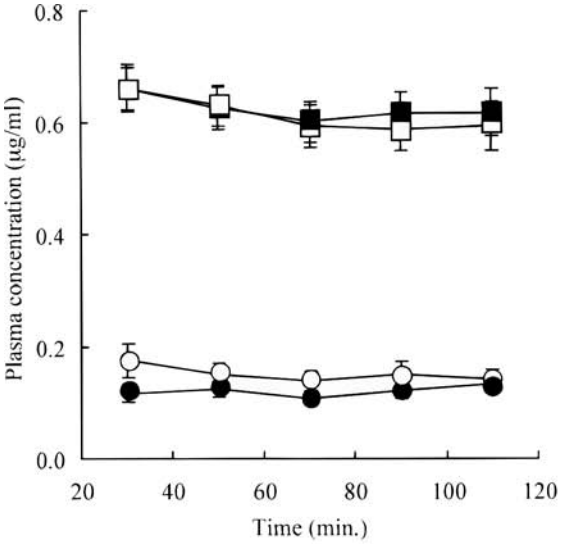

Fig. 6. Time profiles for the plasma concentration of indomethacin and its glucuronide. SD rats (closed symbols) and EHBR (open symbols) received intravenous bolus administration (0.1 mg/kg) followed by constant-rate intravenous infusion (0.3 mg/min/kg for 2 hr) of [^{14}C]indomethacin. The concentrations of indomethacin glucuronide are given as indomethacin equivalent concentrations. Each symbol and vertical bar represents the mean ± S.E. of 5 animals. Squares and circles represent the concentration of indomethacin and its glucuronide, respectively.

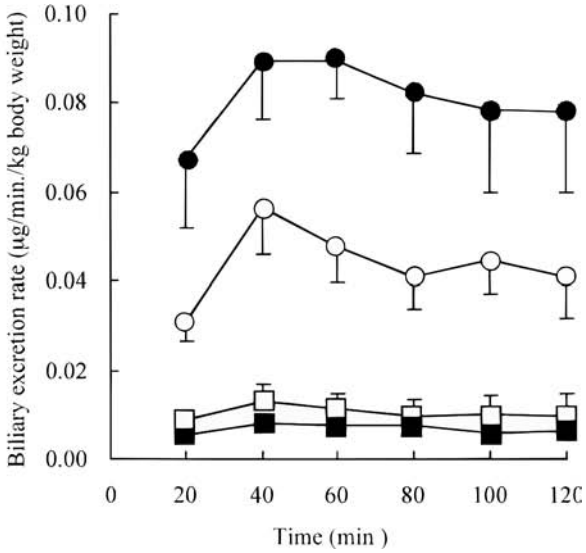

Fig. 7. Time profiles for the biliary excretion of indomethacin and its glucuronide. SD rats (closed symbols) and EHBR (open symbols) received intravenous bolus administration (0.1 mg/kg) followed by constant-rate intravenous infusion (0.3 mg/min/kg for 2 hr) of [^{14}C]indomethacin. The excretion rate of indomethacin glucuronide is given as the indomethacin equivalent rate. Each symbol and vertical bar represents the mean ± S.E. of 5 animals. Squares and circles represent the excretion rate of indomethacin and its glucuronide, respectively.

DISCUSSION

In the present study, we examined the hepatic uptake and biliary excretion mechanisms of indomethacin. For sinusoidal transport, we initially examined the uptake of indomethacin into primary cultured rat hepatocytes. Since it has been reported that the expression of transporters and their function is reduced in hepatocytes cultured for more than 6 hr (22,23), the culture period was restricted to 4 hr or less in the present study (24).

Indomethacin was taken up by primary cultured rat hepatocytes in Na^+-dependent and -independent systems (Fig. 1). The uptake of indomethacin was concentrative, temperature-dependent, and sensitive to a metabolic inhibitor (Fig. 1). Indeed, the uptake in the absence of Na^+ was almost completely abolished at 0°C and in the presence of FCCP (2 μM) (Fig. 1). These results demonstrate that the hepatic uptake of indomethacin is mediated by Na^+-dependent and -independent active transport systems. Most of the hepatic uptake of indomethacin from Krebs-Henseleit buffer is mediated by a saturable component (K_m = 11.5 +/− 6.3 μM) at tracer concentrations (Fig. 2). Moreover, the hepatic uptake of indomethacin (1 μM) was inhibited by TC and non-bile acid organic anions (pravastatin, DBSP and $E_2$17βG) in a concentration-dependent manner (Fig. 3). To reduce the uptake of indomethacin to approximately 50% of the control, 300 μM TC and pravastatin, 100 μM DBSP and 40–70 μM $E_2$17βG were required (Fig. 3). These IC_{50} values should be discussed in relation to their own K_m values. In rat hepatocytes, the K_m values for Na^+-dependent and -independent TC uptake has been reported as 15 and 57 μM, respectively (25). Moreover, the K_m values for Ntcp- and oatp1-mediated TC transport has also been determined as 17 and 50 μM, respectively (17,26). These K_m values are significantly lower than the IC_{50} value of TC for the uptake of indomethacin (~300 μM; Fig. 3). In the same manner, the reported K_m value for $E_2$17βG (13 μM for hepatic uptake and 20 μM for oatp1-mediated uptake (18)) is lower than the IC_{50} value of $E_2$17βG for the uptake of indomethacin (Fig. 3). Moreover, IC_{50} values of pravastatin and DBSP for hepatic and/or oatp1-mediated uptake of $E_2$17βG were approximately 100 and 10 μM, respectively, in our recent study (27) and differ from those in the present study (~300 μM and 100 μM, respectively; Fig. 3). These results are consistent with the hypothesis that the hepatocellular uptake of indomethacin is not predominantly mediated by either Ntcp or oatp1.

This hypothesis was further supported by the results of uptake experiments into cDNA-transfected COS-7 cells. Although the uptake of TC and $E_2$17βG, typical substrates for Ntcp and oatp1, respectively, into COS-7 cells was stimulated by transfecting cDNAs encoding these transporters, the uptake of indomethacin was not affected by cDNA transfection (Figs. 4 and 5). These results suggest that other transporters are responsible for the hepatic uptake of indomethacin. Recently, it has been demonstrated that organic anion transporter (oat1), predominantly located on the basolateral membrane of renal epithelial cells, is responsible for the uptake of indomethacin in exchange for the export of α-ketoglutarate (28). Since it has been demonstrated that oat1 homologues (oat2 and 3) are expressed in the liver (9,10), it may be that these transporters are responsible for the hepatic uptake of indomethacin.

After being efficiently taken up by hepatocytes, it has been demonstrated that indomethacin is metabolized to indomethacin glucuronide, desmethylindomethacin and deschlorobenzoylindomethacin and their glucuronides (29). Since it has been demonstrated that the amount of indomethacin and indomethacin glucuronide excreted in bile is closely related to the intestinal

Table 1. Kinetic Parameters for the Disposition of Indomethacin and its Glucuronide. SD Rats and EHBR Received Intravenous Bolus Administration (0.1 mg/kg) Followed by Constant-Rate Intravenous Infusion (0.3 mg/min/kg for 2 hr) of [^{14}C] indomethacin. Data Shown in Figs. 6 and 7 Were Analyzed to Determine the Kinetic Parameters

	Indomethacin		Indomethacin glucuronide	
	SD	EHBR	SD	EHBR
C_{plasma}(μg/ml)[a]	0.619 ± 0.042	0.594 ± 0.044	0.133 ± 0.012	0.141 ± 0.019
C_{liver} (μg/ml)[b]	0.319 ± 0.021	0.196 ± 0.014**	0.0707 ± 0.0103	0.0663 ± 0.0055
V_{bile} (μg/min/kg body weight)[c]	0.0061 ± 0.0013	0.0099 ± 0.0048	0.078 ± 0.018	0.041 ± 0.009*
CL_{plasma} (ml/min/kg body weight)	0.494 ± 0.045	0.513 ± 0.049	—	—
$CL_{bile,plasma}$ (ml/min/kg body weight)[d]	0.0102 ± 0.0023	0.0169 ± 0.0073	—[f]	—[f]
$CL_{bile,liver}$ (ml/min/kg body weight)[e]	0.0193 ± 0.0041	0.0574 ± 0.0318	1.13 ± 0.21	0.570 ± 0.116*

[a] Measured at 110 min.
[b] Measured at 120 min.
[c] Measured at 100 ~ 120 min.
[d] Calculated by dividing V_{bile} by C_{plasma}.
[e] Calculated by dividing V_{bile} by C_{liver}.
[f] $CL_{bile,plasma}$ for indomethacin glucuronide was not demonstrated, since significant amount of glucuronide is formed in the liver; and therefore, the calculated clearance values are not directly related to the transport activity from blood into bile.
** $P < 0.01$.
* $P < 0.05$ by the paired t-test.

toxicity of this drug (4), we focused on the biliary excretion mechanism of these two molecular species. At steady-state after constant rate infusion of indomethacin, the plasma concentration of indomethacin was almost the same in SD rats and EHBR (Fig. 6), suggesting that the biliary excretion of indomethacin is not predominantly mediated by cMOAT/MRP2 and/or that only a limited amount of indomethacin is excreted into the bile without metabolic conversion even if the excretion is mediated by cMOAT/MRP2. Indeed, it was found that only a limited amount of indomethacin (<3% of the administered dose) was excreted into the bile without metabolic conversion (Fig. 7 and Table 1). Moreover, the biliary excretion rate of indomethacin was almost the same in SD rats and EHBR (Fig. 7 and Table 1). In contrast, approximately 30% of the administered dose of indomethacin was excreted into the bile as its glucuronide (Fig. 7). In addition, the biliary excretion of indomethacin glucuronide in EHBR was significantly lower than that in SD rats (Fig. 7). Kinetic analysis indicated that the $CL_{bile,liver}$ value for indomethacin glucuronide, defined as the rate of excretion into the bile divided by the hepatic concentration, in EHBR was approximately 50% that in SD rats (Table 1). These results indicate that the canalicular export of indomethacin glucuronide is mediated by cMOAT/MRP2 whereas that of indomethacin is not mediated by cMOAT/MRP2. It is plausible that the efficient excretion of indomethacin glucuronide mediated by cMOAT may be related to the gastrointestinal lesions induced by indomethacin.

In conclusion, indomethacin is taken up by rat hepatocytes *via* Na^+-dependent and -independent active transporters which are different from Ntcp and oatp1, respectively. After intravenous infusion, the biliary excretion of indomethacin glucuronide exceeded that of the parent drug. Although the canalicular export of indomethacin was comparable between SD rats and EHBR, it was found that approximately 50% of the glucuronide excretion is mediated by cMOAT/MRP2.

ACKNOWLEDGMENTS

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