Involvement of Human Organic Cation Transporter 1 in the Hepatic Uptake of 1-(2-Methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d]imidazolium Bromide (YM155 Monobromide), a Novel, Small Molecule Survivin Suppressant

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ABSTRACT:

YM155 monobromide, a small molecule that specifically inhibits survivin gene transcription and protein expression in several tumor cell lines (Nakahara et al., 2007). During a phase I clinical study, YM155 exhibited antitumor activity in some patients with non-Hodgkin’s lymphoma, hormone refractory prostate cancer, and non–small-cell lung cancer.

Increasing evidence suggests that survivin, a member of the inhibitor of apoptosis protein family, is an essential regulator of cell division and a modulator of apoptotic cell death (Ambrosini et al., 1997; O’Connor et al., 2000; Giodini et al., 2002; Altieri, 2006). Survivin has been found to be overexpressed in various kinds of tumor tissues (Ambrosini et al., 1997; O’Driscoll et al., 2003), suggesting that survivin may be an attractive, novel target for cancer chemotherapy with the dual aim of preventing cancer cell proliferation and enhancing the cancer cell-selective response to apoptosis.

YM155 monobromide, 1-(2-methoxyethyl)-2-methyl-4,9-dioxo-3-(pyrazin-2-ylmethyl)-4,9-dihydro-1H-naphtho[2,3-d]imidazolium bromide (YM155 monobromide), which is a hydrophilic and cationic compound, exhibits antitumor activity in experimental human hormone refractory prostate carcinoma models. Urinary excretion was 18.3 to 28.6% of the dose in the clinical phase I study, and nonrenal elimination may be explained by the biliary excretion of YM155 in its unchanged form. Because the penetration through the sinusoidal membrane of the hepatocytes is the first step and an important part of biliary excretion, we evaluated the uptake of [14C]YM155 into human cryopreserved hepatocytes. YM155 was taken up into hepatocytes in a temperature- and concentration-dependent manner. The saturable uptake component was much higher than the nonsaturable passive diffusion component. In vitro hepatic uptake clearance was consistent with the in vivo hepatic intrinsic clearance calculated using clinical study data. Hepatic uptake of YM155 was inhibited by organic cation transporter (OCT) inhibitors, and the IC50 values for YM155 uptake were comparable to those reported for human OCT1-mediated transport. The interaction of YM155 with candidate transporter, OCT1, was also characterized using S2 cells stably expressing human OCT1 (OCT1-S2 cells). In OCT1-expressing S2 cells, YM155 inhibited the OCT1-mediated uptake of a typical OCT1 substrate, [14C]tetraethylammonium. In addition, YM155 was taken up into OCT1-S2 cells These results indicated that OCT1 was the predominant transporter for the hepatic uptake of YM155, and the transporter-mediated uptake clearance observed in vitro may account for the in vivo intrinsic hepatic clearance.
(Tolcher et al., 2008). With regard to administration route, YM155 is now being developed as an intravenous formulation.

In a previous study, 18.3 to 28.6% of a dose given to humans as an intravenous continuous infusion was excreted into urine in the unchanged form (Tolcher et al., 2008). In nonclinical studies, YM155 was hardly metabolized when incubated with human cryopreserved hepatocytes (Sohda et al., 2007). In a study conducted in animals, YM155 was eliminated with a high total clearance in dogs (Mine-matsu et al., 2008) and excreted mainly into the bile and urine as the unchanged form, and partly metabolized in rats and dogs (T. Mine-matsu, M. Iwai, K. Y. Sohda, T. Usui, and H. Kaminura, unpublished data). Given these previous findings, the first step in the nonrenal elimination of YM155 (i.e., 71.4–81.7% of the dose) from the human body is likely to be through uptake into hepatocytes from the blood, followed mainly by biliary excretion as the unchanged form and possibly a small amount by metabolism.

Penetration through the sinusoidal membrane of hepatocytes may represent a rate-limiting step for hepatic intrinsic clearance. Organic anion transporting polypeptide (OATP) 1B1, OATP1B3, OATP2B1, organic anion transporter 2, Na–taurocholate cotransporting polypeptide, and organic cation transporter (OCT) 1 are known to be localized on the basolateral membrane of hepatocytes and mediate the hepatic uptake of their substrates from circulating blood (Gründemann et al., 1994; Hageman and Meier, 1996; Hsiang et al., 1999; Faber et al., 2003). OCT3 and OATP1A2 mRNA have also been detected in the liver (Kullak-Ublick et al., 1995; Gründemann et al., 1998).

The effects of hepatic uptake transporters on the pharmacokinetics of their substrates have been well characterized for anionic drugs, particularly HMG CoA reductase inhibitors (statins) (Maeda and Sugiyama, 2008). However, despite the knowledge that several hydrophilic cationic drugs act as a substrate for OCT1, few studies have reported on the effects of OCT1 on the capacity for hepatic clearance, because hydrophilic cationic drugs tend to be excreted into urine rather than bile. Metformin, a drug widely used to treat type 2 diabetes, is known to be a substrate of both OCT1 and OCT2 (Zhang et al., 1997; Wang et al., 2002). Although metformin is extensively eliminated from the kidney in humans, probably by OCT2, [renal clearance accounts for 78.9–99.9% of the total clearance (Scheen, 1996)], its pharmacological effects, which are mediated by distribution into hepatocytes, and pharmacokinetics after oral administration were both altered by a reduction in OCT1 function due to genetic variation (Shu et al., 2007, 2008). Furthermore, Umehara et al. (2007) reported that the saturable uptake of 1-methyl-4-phenylpyridinium (MPP), tetraethylammonium (TEA), cimetidine, and metformin into human and rat hepatocytes was highly similar to that into human and rat OCT1-expressing HEK293 cells. These observations indicate that, in humans and rats, OCT1-mediated uptake accounts for most of the saturable hepatic uptake of organic cations tested, whereas nonsaturable passive diffusion was found to be the major component (except for MPP). Based on these findings, OCT1 is thought to play a predominant role in the saturable hepatic uptake of cationic compounds, although the fraction of nonsaturable component should be considered.

In this study, we elucidated the hepatic uptake mechanism of YM155, a certain amount of which is expected to be eliminated via the bile. Uptake of [14C]YM155 was characterized using human cryopreserved hepatocytes and various transporter inhibitors, and the in vitro hepatic uptake clearance value obtained was compared with the in vivo hepatic intrinsic clearance estimated from a previous clinical study. Involvement of the transporter was confirmed using cells transfected to express the candidate transporter, OCT1, derived from the second portion of the proximal tubes (S2 cells).

Materials and Methods

Materials. [14C]YM155 monobromide (specific activity, 3.05 MBq/mg; radiochemical purity, 98.9%) was synthesized at Sekisui Medical Co. Ltd. (Ibaraki, Japan). YM155 monobromide (assay value, 100.1%) was synthesized at Astellas Pharma Inc. (Tokyo, Japan). [14C]TEA (specific activity, 2.035 GBq/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). All other chemicals and reagents used were commercially available and of guaranteed purity.

Cell Culture. OCT1-S2 cells and mock-transfected (control) cells established as described previously (Takeda et al., 2002) were used for 19 to 34 passages. The full-length cDNA of OCT1 was subcloned into pcDNA3.1 (Invitrogen, Carlsbad, CA), and S2 cells were transfected via this vector using Tfx-50 (Promega, Madison, WI). S2 cells transfected via an empty vector (mock-S2 cells) were used as the control. These cells were cultured in the RITC 80-7 medium, containing 9.83 g/l RITC 80-7 basic medium (Iwaki, Tokyo, Japan), 3.3 g/l HEPES, 1.4 g/l NaHCO3, 10 mg/l transferrin, 80 unit/l insulin, 50 ml/l fetal bovine serum, 10 µg/l EGF, and 400 mg/l genicic sulfate (Wako, Osaka, Japan), at 33°C in an atmosphere of 5% CO2 in humidified air.

In Vitro Uptake Experiments. Uptake study using human cryopreserved hepatocytes. The uptake study was performed as described previously (Shitara et al., 2003a). Three lots of human cryopreserved hepatocytes (lot 453, lot 470, and lot 466) were purchased from Xenotech, LLC (Lenexa, KS) and stored in a liquid nitrogen unit before use. HEPES Krebs-Henseleit buffer, consisting of 11.1 mM Na3HPO4, 1.17 mM MgSO4, 1.18 mM KH2PO4, 4.69 mM KCl, and 143 mM HEPES, was prepared and adjusted to pH 7.4. Cryopreserved hepatocytes were thawed per the manufacturer’s instruction using a Hepatocyte Isolation Kit (Xenotech, LLC), and resuspended in HEPES Krebs-Henseleit buffer (pH 7.4) at a cell density of 2.0 x 107 viable cells/ml. Before starting the [14C]YM155 uptake experiments, the cell suspensions (110 µl) were prewarmed in a 37°C water bath or precooled in ice water for 3 min. The uptake of [14C]YM155 was initiated by adding an equal volume of prewarmed or precooled [14C]YM155 solution to yield a final viable cell concentration of 1.0 x 106/ml. [14C]YM155 concentrations, uptake time, and inhibitor concentrations, if any, were described in each experiment. Uptake was stopped by separating the cells from the [14C]YM155 solution, after which the mixture was incubated for a designated time period. For this purpose, an aliquot of the incubation mixture was transferred to a 400-µl polyethylene tube containing 50 µl of NaOH (2 M) under a 100-µl layer of an oil mixture. The sample tube was then centrifuged briefly. After overnight incubation in alkaline to dissolve the hepatocytes, the centrifuge tube was cut into two compartments: one containing the [14C]YM155 solution (component M), and the other containing the dissolved cells (component C). After the addition of 10 ml of Hionic Fluor, the amount of uptake was then determined by measuring the radioactivity in both the cells and the solution using a liquid scintillation counter.

Uptake study using OCT1-S2 cells. Two days before the uptake experiments, the cells were seeded in 24-well cell culture dishes at a density of 1 x 105 cells/well. The cells were preincubated for 10 min at 37°C in Dulbecco’s phosphate-buffered saline (DPBS; pH 7.4), which consisted of 137 mM NaCl, 3 mM KCl, 8 mM NaHPO4, 1 mM KH2PO4, 1.18 mM MgSO4, 4.69 mM KCl, and 143 mM NaHCO3, 1.17 mM MgSO4, 1.18 mM KH2PO4, 4.69 mM KCl, and 143 mM HEPES, was prepared and adjusted to pH 7.4. Cryopreserved hepatocytes were thawed per the manufacturer’s instruction using a Hepatocyte Isolation Kit (Xenotech, LLC), and resuspended in HEPES Krebs-Henseleit buffer (pH 7.4) at a cell density of 2.0 x 107 viable cells/ml. Before starting the [14C]YM155 uptake experiments, the cell suspensions (110 µl) were prewarmed in a 37°C water bath or precooled in ice water for 3 min. The uptake of [14C]YM155 was initiated by adding an equal volume of prewarmed or precooled [14C]YM155 solution to yield a final viable cell concentration of 1.0 x 106/ml. [14C]YM155 concentrations, uptake time, and inhibitor concentrations, if any, were described in each experiment. Uptake was stopped by separating the cells from the [14C]YM155 solution, after which the mixture was incubated for a designated time period. For this purpose, an aliquot of the incubation mixture was transferred to a 400-µl polyethylene tube containing 50 µl of NaOH (2 M) under a 100-µl layer of an oil mixture. The sample tube was then centrifuged briefly. After overnight incubation in alkaline to dissolve the hepatocytes, the centrifuge tube was cut into two compartments: one containing the [14C]YM155 solution (component M), and the other containing the dissolved cells (component C). After the addition of 10 ml of Hionic Fluor, the amount of uptake was then determined by measuring the radioactivity in both the cells and the solution using a liquid scintillation counter.

Data analysis and estimation of kinetic parameters. Uptake into human cryopreserved hepatocytes was given as the cell-to-medium ratio (C/M), which was determined as the radioactivity in component C divided by that in component M.
component M. Kinetic analysis of the concentration-dependent uptake of YM155 into hepatocytes was performed using WinNonlin version 4.1 (Pharsight Corporation, Mountain View, CA). The following equations (eqs. 1 and 2) were simultaneously fit to the substrate concentration ([S]) versus uptake rate (V, expressed as pmol/min/10^6 cells), which was determined as C/M divided by uptake time and multiplied by 60.

For 37°C incubation:

\[ V = \frac{V_{\text{max}} \cdot [S]}{K_m + [S]} + P_{\text{dif}} \cdot [S] \]  

(1)

For on-ice incubation:

\[ V = P_{\text{dif}} \cdot [S] \]  

(2)

where \( P_{\text{dif}} \) is component of uptake clearance by passive diffusion, adsorption to cell surface, and adhering medium. To compare in vitro transporter-mediated hepatic uptake clearance with in vivo nonrenal clearance in humans, in vitro transporter-mediated intrinsic uptake clearance (PS_{\text{intrinsic, in vitro}}) was calculated as \( V_{\text{max}}/K_m \). The physiological parameters used for scaling PS_{\text{intrinsic, in vitro}} from “pmol/min/10^6 cells” to “L/h (hr)/person” are described in Table 1.

For OCT1-S2 cells, the \( K_m \) and \( V_{\text{max}} \) values were calculated by the following equation. [14C]YM155 uptake via OCT1 (V) was calculated by subtracting uptake in mock cells from that in OCT1-S2 cells.

\[ V = \frac{V_{\text{max}} \cdot [S]}{K_m + [S]} \]  

(3)

In the inhibition study using hepatocytes, carrier-mediated uptake was calculated by subtracting the C/M on ice from that at 37°C. The C/M on ice used for the calculation shown in Fig. 3 was measured only when no organic solvent or inhibitor was present, whereas the C/M on ice values shown in Fig. 4 were obtained for each inhibitor concentration. Each carrier-mediated uptake value was normalized using data from the control experiments (percentage of control). The IC_{50} values were calculated using two points for the logarithm of concentrations versus uptake (percentage of control) at approximately 50% inhibition (Minematsu et al., 2009).

In the inhibition study using OCT1-S2 cells, mode of inhibition (competitive or noncompetitive) was determined by Lineweaver-Burk plot analysis, and the \( K_i \) values were calculated from the following equation:

\[ K_i = \frac{[I]}{K_m \cdot \text{inhibitor}} \left( \frac{K_m \cdot \text{inhibitor}}{K_m \cdot \text{inhibitor}} \right) - 1 \]  

(4)

where \([I]\) is inhibitor concentration, and \( K_m \cdot \text{inhibitor} \) and \( K_m \cdot \text{inhibitor} \) are the \( K_m \) values of substrate compound with and without inhibitor, respectively.

In Vivo Pharmacokinetic Data Analysis. The values for the fraction of unchanged YM155 excreted into urine (Fe) and total clearance calculated from the plasma YM155 concentrations (CL_{int}) after continuous intravenous infusion at 1.8, 3.6, and 4.8 mg/m^2/day to cancer patients were derived from a previous study (Tolcher et al., 2008). Nonrenal clearance based on the blood drug concentration (CL_{nr}) in vivo was calculated as follows; \( CL_{nr} = (1-Fe) \cdot CL_{int}/R_B \), where \( R_B \) is the YM155 concentration ratio of blood to plasma. An \( R_B \) value of 0.63, which was obtained from the in vitro experiments (T. Minematsu, N. Shirai, T. Usui, and H. Kamimura, unpublished data), was used. Assuming that nonrenal clearance represents hepatic clearance, hepatic intrinsic clearance in vivo (CL_{int \text{in vivo}}) was subsequently calculated from the following equation using the well stirred model (eq. 5) and dispersion model (eqs. 6 and 7):

\[ CL_{nr} = \frac{Q_B \cdot fp \cdot CL_{int \text{in vivo}}}{Q_B + fp \cdot CL_{int \text{in vivo}}} \]  

(5)

\[ CL_{nr} = \frac{Q_B \left( 1 + \frac{4a}{(a-1)^2} \exp \left( \frac{a-1}{2D_n} \right) - \frac{1}{(a+1)^2} \exp \left( \frac{a+1}{2D_n} \right) \right) }{R_B \cdot Q_B} \]  

(6)

\[ a = \left( 1 + \frac{4fp \cdot CL_{int \text{in vivo}} \cdot D_n}{R_B \cdot Q_B} \right)^{1/2} \]  

(7)

where \( Q_B \) is hepatic blood flow, with a value of 1450 mL/min/70 kg (87 L/h/70-kg person) (Davies and Morris, 1993), and \( fp \) is the plasma unbound fraction, with a value of 0.81 (obtained from the in vitro experiments; T. Minematsu, T. Usui, and H. Kamimura, unpublished data). \( D_n \) is the dispersion number, for which a value of 0.17 was used.

Results

Time Courses of the Uptake of [14C]YM155 into Human Cryopreserved Hepatocytes. The time courses for the uptake of [14C]YM155 into human cryopreserved hepatocytes are shown in Fig. 1. It is known that a large interbatch difference in cryopreserved hepatocyte uptake activity for the same compounds could be observed, probably due to differences in hepatocyte isolation and cryopreservation conditions, as well as interindividual variability in the expression and function of the transporters (Shitara et al., 2003b). The present study was conducted using three batches of human cryopreserved hepatocytes prepared from three independent donors. The C/M ratio at 37°C was much greater than that on ice for all hepatocytes lots, suggesting an uptake of radioactivity into the cells. Linear uptake was observed for at least 5 min for lots 453 and 466 and 3 min for lot 470.

Concentration-Dependent Uptake of [14C]YM155 into Human Cryopreserved Hepatocytes. Concentration dependence of [14C]-YM155 uptake using three different lots of human hepatocytes is shown in Fig. 2. Uptake was examined 5 min after initiation of uptake for lots 453 and 466, and 3 min after for lot 470. The saturable, concentration-dependent kinetics were successfully described by assuming the presence of one saturable and one linear passive component at 37°C, but only a linear passive component on ice (Table 1). The \( K_m \) values for YM155 among the three lots of hepatocytes were comparable and ranged from 6.13 to 9.47 \( \mu \)M. In a phase I clinical study, the mean steady-state plasma concentration of YM155 was 21 nM at the maximum tolerated dose (4.8 mg/m^2/day) (Tolcher et al., 2008). Therefore, because YM155 has been developed as intravenous dose formulation, transporter-mediated uptake may be expected to be linear under clinical conditions. Furthermore, whereas the \( V_{\text{max}} \) values varied from 29.3 to 87.3 pmol/min/10^6 cells, the transporter-mediated uptake was much greater than that on ice for all hepatocytes lots, probably due to differences in hepatocyte isolation and cryopreservation conditions. LPIC values were determined by Lineweaver-Burk plot analysis, and the \( K_i \) values were calculated from the following equation using the well stirred model (eq. 5) and dispersion model (eqs. 6 and 7):

\[ CL_{nr} = \frac{Q_B \cdot fp \cdot CL_{int \text{in vivo}}}{Q_B + fp \cdot CL_{int \text{in vivo}}} \]  

(5)

\[ CL_{nr} = \frac{Q_B \left( 1 + \frac{4a}{(a-1)^2} \exp \left( \frac{a-1}{2D_n} \right) - \frac{1}{(a+1)^2} \exp \left( \frac{a+1}{2D_n} \right) \right) }{R_B \cdot Q_B} \]  

(6)

\[ a = \left( 1 + \frac{4fp \cdot CL_{int \text{in vivo}} \cdot D_n}{R_B \cdot Q_B} \right)^{1/2} \]  

(7)
intrinsic clearance ($V_{\text{max}}/K_m$) values (4.22–9.77 μl/min/10⁶ cells, or 54.7–126.5 l/h/person) were much higher than the nonsaturable component of hepatic uptake clearance ($P_{\text{diff}}$ value), accounting for 73 to 85% of total uptake clearance ($V_{\text{max}}/K_m + P_{\text{diff}}$).

**Comparison of In Vitro Hepatic Uptake Clearance and in Vivo Hepatic Intrinsic Clearance.** Parameters obtained by in vitro study were compared with the $\text{CL}_{\text{H int}}$ in vivo, which was calculated using the well stirred and dispersion models (Table 2). The $\text{CL}_{\text{H int}}$ in vivo values obtained were 39.2 to 95.0 l/h/person using the well stirred model, and 32.9 to 66.8 l/h/person using the dispersion model, at a dose of 1.8 to 4.8 mg/m²/person. These values were similar to the range observed for in vitro transporter-mediated uptake clearance (54.7–126.5 l/h/person) (Table 2). Furthermore, the fp/$R_B$ · $\text{CL}_{\text{H int}}$ in vivo values were comparable with the $Q_{\text{H}}$ value (87.0 l/h/person).

**Effects of Various Compounds on the Uptake of [¹⁴C]YM155 into Human Cryopreserved Hepatocytes.** The effects of various compounds on the uptake of [¹⁴C]YM155 into human cryopreserved hepatocytes are shown in Fig. 3. [¹⁴C]YM155 uptake was strongly

**Fig. 1.** Time course of cell uptake to medium concentration ratio (C/M) of [¹⁴C]YM155 (10 μM) by human hepatocytes lot 453 (A), lot 466 (B), and lot 470 (C). Each point represents the mean of duplicate experiments. Uptake was examined at 37°C (●) and on ice (○).

**Fig. 2.** Eadie-Hofstee plot for [¹⁴C]YM155 uptake into human cryopreserved hepatocytes lot 453 (A), lot 466 (B), and lot 470 (C). Uptake was examined at 5 min after the initiation of uptake for lots 453 and 466 and 3 min after for lot 470. Each point represents the mean ± S.D. of three independent experiments. The solid line is the line of best-fit. Uptake was examined at 37°C (●) and on ice (○).
inhibited by MPP, verapamil, amantadine, procainamide, corticosterone, prazosin, and cimetidine, and moderately inhibited by TEA, probenecid, and K-strophanthin. In contrast, no marked inhibition was observed in the presence of 1-methylnicotinamide and taurocholate. Detailed concentration-dependent inhibition profiles obtained using corticosterone, MPP, prazosin, and cimetidine as inhibitors are shown in Fig. 4. These compounds were selected due to their strong inhibitory effects on YM155 uptake (Fig. 3) and variable affinity for the OCT candidate transporters. As shown in Table 3, the IC50 values for corticosterone, MPP, prazosin, and cimetidine were 4.70, 16.7, 1.43, and 147 μM, respectively, which were consistent with the reported values for OCT1 (Koepsell et al., 2003, 2007). YM155 and cimetidine were 4.70, 16.7, 1.43, and 147 μM, respectively, which were consistent with the reported values for OCT1 (Koepsell et al., 2003, 2007). YM155 inhibited OCT1-mediated uptake in a concentration-dependent manner. The IC50 value of YM155 was calculated as 22.5 μM, indicating some interaction between YM155 and OCT1. Analysis of the inhibitory mechanism via the Lineweaver-Burk plot revealed that Vmax of [14C]TEA in the absence or presence (30 μM) of YM155 were 509.4 and 469.2 pmol/min/mg protein, respectively, showing no significant change, whereas Km values increased from 566.2 μM in the absence of YM155 to 826.0 μM in the presence of YM155 (Fig. 5B). The inhibition of OCT1-mediated uptake by YM155 was therefore determined to be competitive, and the Km value of YM155 was estimated to be 65.4 μM. In addition, the time-dependent uptake of [14C]YM155 into OCT1-S2 cells is shown in Fig. 6A. Figure 6B shows the concentration dependence of OCT1-mediated [14C]YM155 uptake after 5 min of incubation. The uptake exhibited saturable kinetics. The Km and Vmax values were estimated to be 38.7 μM and 103.9 pmol/min/mg protein, respectively.

### Discussion

In this study, our examination of the uptake of YM155 into human cryopreserved hepatocytes and OCT1-S2 cells showed that YM155 was taken up in a carrier-mediated manner. IC50 values of organic cations for YM155 transport were consistent with those reported for OCT1. Results from the present study suggest that the in vitro hepatic uptake clearance of YM155 may reflect in vivo clearance well in humans, and they also indicate the importance of OCT1-mediated transport in elimination of YM155 via bile.

In the earlier phase I study, intravenous continuous infusion of YM155 resulted in 18.3 to 28.6% of the dose being excreted into urine in the unchanged form (Tolcher et al., 2008). Considering the nonclinical study results, nonrenal elimination of YM155 from the body (specifically, 71.6–81.7% of the dose) is likely to start with uptake from the blood into hepatocytes, followed mainly by excretion of the unchanged form via the biliary pathway, where it has only a small possibility of being metabolized.

In the present study, the uptake of [14C]YM155 was characterized using three lots of human cryopreserved hepatocytes. [14C]YM155 was taken up into the hepatocytes in a time- and incubation temperature-dependent manner (Fig. 1). Our concentration-dependent uptake study revealed that the kinetics were successfully described by a one saturable-component model. The Km values (6.13–9.47 μM) for YM155 were comparable among the three lots of hepatocytes. Furthermore, the saturable, or transporter-mediated, intrinsic clearance values (Vmax/Km) of YM155 were much larger than those for passive diffusion (Papp). Given these findings, hepatic uptake transporters are expected to play an important role in the uptake of YM155 under pharmacologically effective concentrations, unlike the hepatic uptake of metformin and cimetidine, most of which is achieved through passive diffusion (Umehara et al., 2007).

For YM155, nonrenal clearance can be used as a substitute parameter for the evaluation of hepatic clearance. Focusing on the human study, the fp/RH · CLH int in vivo value calculated was comparable with the Qs value, hence both the CLH int in vivo, and Qs values were important factors affecting the hepatic elimination of YM155. In general, hepatic intrinsic clearance can be described as follows:

\[
CL_{H\text{ int in vivo}} = \frac{PS_{\text{uptake, basal}} \cdot PS_{\text{eff, apical}} + CL_{\text{metab}}}{PS_{\text{eff, apical}} + CL_{\text{metab}} + PS_{\text{eff, basal}}} \tag{8}
\]

where PS_{uptake, basal} is the uptake clearance via the sinusoidal membrane of the hepatocytes, PS_{eff, apical} is efflux clearance via the apical membrane into the bile, CL_{metab} is metabolic clearance, and PS_{eff, basal} is the efflux clearance via the sinusoidal membrane of the hepatocytes. For YM155, CL_{metab} may be neglected, because YM155 is expected to be metabolized very little, if at all.

With regard to hepatic metabolic clearance, many approaches have

### TABLE 2

**Summary of in vivo hepatic intrinsic clearance of YM155**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dose Level (mg/m²/day)</th>
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<tr>
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<tr>
<td>CL_{H\text{ int in vivo}} (l/h/person)</td>
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<td>Well stirred model</td>
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<td>CL_{H\text{ int in vivo}} (l/h/person)</td>
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<td>fp/RH · CL_{H\text{ int in vivo}} (l/h/person)</td>
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<td>Dispersion model</td>
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<td>CL_{H\text{ int in vivo}} (l/h/person)</td>
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<tr>
<td>fp/RH · CL_{H\text{ int in vivo}} (l/h/person)</td>
<td>42.3</td>
</tr>
</tbody>
</table>

*These data were calculated from the data cited from Tolcher et al., 2008.
been reported for comparing the CL_H int in vitro obtained from in vitro experiments with the CL_H int in vivo, calculated from in vivo pharmacokinetic data, and for predicting CL_H int in vivo from CL_H int in vitro (Izumi et al., 1997; Naritomi et al., 2003; Huang et al., 2008). However, the usefulness of human cryopreserved hepatocytes for the prediction of P_{\text{uptake},\text{basal}} has not been fully confirmed yet (Watanabe et al., 2009). In the present study, the P_{\text{uptake},\text{basal}} values estimated from hepatocyte uptake study were able to reflect P_{\text{uptake},\text{basal}} values in vitro (Table 1; eq. 8). On comparison of in vitro and in vivo data, P_{\text{uptake},\text{basal}} values were found to be consistent with CL_H int in vitro values (Table 2). Furthermore, with the scaling factor from in vitro to in vivo of P_{\text{uptake},\text{assumed}} to be 1, P_{\text{eff},\text{apical}} is estimated to be much higher than P_{\text{eff},\text{basal}} according to eq. 8. It may indicate the involvement of efflux transporters, such as P-glycoprotein and/or multidrug and toxin extrusion 1, which transport organic cations and are expressed in the apical membrane of the hepatocytes in the clinical condition (Hoffmann and Kroemer, 2004; Otsuka et al., 2005).

To determine the uptake transporter(s) engaged in the hepatic uptake of YM155, inhibitory effects of various cations and anions on YM155 uptake were characterized. YM155 uptake was found to be inhibited by OCT family inhibitors such as TEA, MPP, probenecid, verapamil, amantadine, procainamide, corticosterone, prazosin, and cimetidine (Fig. 3). In contrast, no significant inhibition was observed in the presence of taurocholate, an inhibitor of OATPs. Probenecid, known to inhibit OATs, and K-strophanthin, known to inhibit OATP1A2, partially inhibited YM155 uptake. Because these compounds also inhibit OCT at high concentrations (Arndt et al., 2001; van Montfoort et al., 2001), the inhibitions caused by the latter two may be due to the inhibition of OCT-mediated YM155 transport rather than OAT- or OATP-mediated transport. At present, three types of OCTs (OCT1, OCT2, and OCT3) have been well identified (Koepsell et al., 2003). Among them, OCT1 and OCT3 are expressed in the human liver, where OCT2 is mainly expressed in the kidney (Koepsell et al., 2003). Potential involvement of OCT2 in the hepatic uptake of YM155 was ruled out on observation that 1-methylnicotinamide, which should inhibit only OCT2 at 1000 μM, did not inhibit YM155 uptake into human cryopreserved hepatocytes, whereas prazosin, which inhibits OCT1 and OCT3 but not OCT2 (Koepsell et al., 2003), completely inhibited YM155 uptake. Taken together, these findings indicate the involvement of either or both OCT1 or OCT3.

To achieve detailed characterization, an inhibition profile was evaluated using corticosterone, MPP, prazosin, and cimetidine as inhibitors. Although the hepatic expression level of OCT3 is low, OCT1 and OCT3 may be due to the inhibition of OCT-mediated YM155 transport. Moreover, the affinity of several organic cations, including the substances or inhibitors that have been identified for either of these transporters, but the affinity of several organic cations, including the inhibitors in this study, does differ between the two (summarized in Table 3). The comparison of a combination of organic cation IC_{50} values may thus aid in clarifying which OCT subtype predominantly contributes to the hepatic uptake of YM155.

With regard to YM155 uptake into human cryopreserved hepatocytes, the IC_{50} values of all tested cationic compounds were similar to those for OCT1, but not for OCT3 (Table 3). Although the reported IC_{50} values were estimated using substrates other than YM155, the

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**TABLE 3**

Summary of the IC_{50} values obtained from YM155 transport and information from the literature on the inhibitory effects of corticosterone, MPP, prazosin, and cimetidine on OCTs

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} Values from the Literature a, b</th>
<th>OCT1</th>
<th>OCT2</th>
<th>OCT3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosterone</td>
<td>4.70, 7.22/100 30 100</td>
<td>34</td>
<td>0.12</td>
<td>0.29</td>
</tr>
<tr>
<td>MPP</td>
<td>16.7, 12/50 200 1000</td>
<td>2.4</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Prazosin</td>
<td>1.43, 1.8/100 30</td>
<td>&gt;100</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>147, 166/50 2000</td>
<td>70</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

a The data shown in Fig. 4 were analyzed to determine IC_{50} values.
b Data from the literature were also summarized for comparison.
similar inhibition profile observed suggests that OCT1, rather than OCT3, plays an important role in the hepatic uptake of YM155.

The interaction between YM155 and OCT1 was further confirmed by experiments using OCT1-expressing S2 cells. YM155 inhibited the OCT1-mediated uptake of TEA with an IC50 value of 22.5 µM, and the uptake of [14C]YM155 into OCT1-S2 cells was much higher than that observed in control cells (Km value of 38.7 µM). The Km value was slightly higher than that obtained in hepatocytes (6.13–9.47 µM).

Considering the combination of organic cation IC50 values, the difference in the two systems (such as the expression systems of OCT1-S2 and the hepatocytes) and not the involvement of other transporters may have contributed to the difference in Km values (Umehara et al., 2007). The discrepancy may have been caused by variations in microenvironment, post-transcriptional modifications, splicing variants, among other differences.

Previous reports conducted in PC-3-xenografted nude mice have demonstrated that the radioactivity of [14C]YM155 is highly distributed into tumors, the site of pharmacological activity, during subcutaneous infusion (Nakahara et al., 2007). Furthermore, YM155 uptake experiments using a range of cancer cell lines such as PC-3, Calu-6, NCI-H358, A375, SK-MEL-5, RL (Minematsu et al., 2009), and Ramos cells indicated the existence of a carrier-mediated uptake process. However, the uptake mechanisms of YM155 involved in cancer cells are considered to be probably different from those in hepatocytes. Although the Km values were similar among these cancer cell lines (0.189–0.367 µM), the values were much lower than those obtained from human cryopreserved hepatocytes and OCT1-S2 cells. In addition to the difference in Km uptake values, the inhibitory profile and IC50 values also differed from these for OCT1, indicating that cancer cells and hepatocytes use different mechanisms to take up YM155.

In humans, YM155 is also excreted into the urine as the unchanged form (Tolcher et al., 2008). OCT1 is predominantly expressed in liver, and its expression, although detectable using reverse transcriptase polymerase chain reaction, was considered low in other tissues, including the kidney (Gorboulev et al., 1997). Taking this into consideration, the uptake of YM155 into the proximal tubule appeared to be mediated by the transporter(s) other than OCT1.

In conclusion, we characterized the uptake of YM155 into human cryopreserved hepatocytes and OCT1-S2 cells. YM155 was taken up into hepatocytes in a carrier-mediated manner. Detailed analyses revealed that the IC50 values of organic cations for YM155 transport were consistent with those reported for OCT1. Taking into account the inhibitory profile of OCT1 and comparing its Km values to those obtained in OCT1-S2 cells, the hepatic uptake of YM155 appears to be mediated by OCT1. In addition, the PSuptake,in vitro values obtained from in vitro data were similar to the CLH int in vivo values. These
results suggest that the hepatic uptake clearance of YM155 in vitro may reflect hepatic intrinsic clearance in vivo well in humans, and that one of the important factors for elimination of YM155 via bile is OCT1-mediated transport. However, urinary excretion and high distribution into tumor tissues are probably mediated by other transporters.

References

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