Survivin is a member of the “inhibitors of apoptosis” family of proteins that has been implicated in both the preservation of cell viability and the regulation of mitosis in tumor cells (Rothe et al., 1995; Roy et al., 1997; Ja¨a¨ttela ¨, 1999; Verdecia et al., 2000). Survivin is undetectable in most normal differentiated tissues, with the exception of the normal placenta, the testes, and rapidly dividing cells such as CD34
/H11001

bone marrow stem cells (Ambrosini et al., 1997; Lu et al., 1998; Tamm et al., 1998; O’Connor et al., 2000). The high expression of survivin in tumors has been correlated with the more aggressive form of neuroblastoma (Adida et al., 1998; Islam et al., 2000) and breast cancer (Tanaka et al., 2000). Given its preferential expression in tumor cells, its ability to block apoptosis and to regulate cancer cell proliferation as well as its correlation with poor survival, survivin seems to be an attractive novel target for cancer therapy. Suppression of survivin renders cells sensitive to normal cell cycle regulation and induces tumor cell death (Giodini et al., 2002). It may also make cells responsive to apoptosis induced by other chemotherapeutic agents (Yamamoto and Tanigawa, 2001).

YM155 monobromide (Fig. 1) inhibits survivin expression in tumor cells (Nakahara et al., 2007). YM155 has been shown to exert inhibitory effects on cell growth in a variety of human tumor cell lines (Nakahara et al., 2007). Moreover, in nude mice carrying the human hormone refractory prostate cancer, PC-3 tumors, 3-day continuous
subcutaneous infusion of YM155 (3–10 mg/kg) induced massive tumor regression accompanied by suppression of intratumoral survivin (Nakahara et al., 2007). In a phase I study conducted in the United States to investigate the safety and tolerability of YM155 7-day continuous intravenous administration every 3 weeks, three of five patients with non-Hodgkin’s lymphoma showed an objective response at doses of 5 to below the maximum tolerated dose (Tolcher et al., 2008). Two patients with hormone-refractory prostate cancer exhibited a prostate-specific antigen response (>50% reduction) and one patient with non–small-cell lung cancer exhibited a minor response (23% reduction), based on Response Evaluation Criteria in Solid Tumors (Tolcher et al., 2008).

A previous study in nude mice bearing PC-3 xenografts reported that YM155, administered as a subcutaneous infusion reached maximum concentrations in the tumor that were approximately 20-fold higher than those in the plasma (Nakahara et al., 2007), which suggests a specific mechanism for this high distribution. However, the concentration of YM155 in organs other than tumors has not been measured, nor has there been any detailed characterization of the uptake of YM155 into the tumor. In general, membrane transporters have been found to play important roles in the distribution of anticancer drug, and in the chemosensitivity and chemoresistance of cancer cells (Huang and Sadeé, 2006). There have been many studies both on ATP-binding cassette transporters that cause the efflux of anticancer drugs from the cancer cells and the role of influx transporters in the uptake of anticancer drugs that are structurally related to endogenous substances and nutrients (Huang and Sadeé, 2006). However, reports on the other influx mechanisms for anticancer drugs that are not analogs of endogenous substances have been limited. Several recent studies have suggested that organic cation influx transporters may contribute to the accumulation and specificity of anticancer drugs to cancer cells (e.g., organic cation transporter 6 (OCT6/SLC22A16) for doxorubicin (Okabe et al., 2005), OCT1 (SLC22A1) for imatinib (Thomas et al., 2004; Wang et al., 2008), and OCT1 and OCT2 (SLC22A2) for oxalatin (Yonezawa et al., 2006; Zhang et al., 2006)). Although YM155 is in fact a substrate of human OCT1 (Minematsu et al., 2007), the role that OCT1 plays in the uptake of YM155 into cancer cells is unknown.

In this study, whole body radioluminography was performed to assess the tissue (tumor and other tissues) distribution of radioactivity after administration of [14C]YM155 to nude mice bearing PC-3 xenografts. In addition, the in vitro uptake of [14C]YM155 into a number of solid- and hematological tumor cell lines was characterized. The possible role of OCT1 in the uptake of YM155 into the tumor cells is also discussed.

Materials and Methods

Materials. [14C]YM155 monobromide (specific activity, 3.05 and 3.27 MBq/mg as monobromide; radiochemical purity not less than 98.2%) was synthesized at Sekisui Medical Co. Ltd. (Ibaraki, Japan). YM155 monobromide (molecular weight, 443.29; assay value, 100.1%) was synthesized at Astellas Pharma Inc. The dose level and concentrations were expressed as YM155, the cationic moiety of this drug substance. All other chemicals and reagents used were commercially available and of guaranteed purity.

Cell Lines. Cell lines derived from human hormone-refractory prostate adenocarcinoma (PC-3), lung cancer (Calu-6 and NCI-H358), melanoma (A375 and SK-MEL-5), and non-Hodgkin’s lymphoma (RL and Ramos) were purchased from American Type Culture Collection (Manassas, VA). PC-3, Calu-6, NCI-H358, A375, SK-MEL-5, all of which have an adherent growth property, were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum at 37°C in a humidified atmosphere of 5% CO2, and subcultured using trypsin EDTA. RL and Ramos, which have a nonadherent growth property, were cultured in RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum, at 37°C in an atmosphere of 5% CO2 in humidified air, and subcultured by diluting the cell culture medium.

In Vivo Distribution of [14C]YM155 in PC-3 Xenograft Nude Mice Using Whole Body Radioluminography. Five-week-old male nude mice (BALB/c-nu/nu) were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). The mice were kept in a cage at 23 to 26°C and a relative humidity of 46 to 56%. The mice were given pellet diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and water (well water sterilized by admixing with sodium hypochlorite; residual chlorine concentration, approximately 2 ppm) ad libitum during the acclimation and experiment periods. After acclimation for more than 5 days, PC-3 cells (3 × 106 cells/animal) were subcutaneously injected into the right dorsum near the forelimbs of the mice and allowed to grow for 24 days to obtain a tumor volume of >250 mm3 (length × width2 × 0.5). [14C]YM155 was continuously infused, subcutaneously, at the rate of 3 mg/kg/day (12 MBq/kg/day) for 3 days to 2 mice using an implanted microosmotic pump (Alzet model 1003D; Durect Corporation, Cupertino, CA). YM155 induced massive tumor regression accompanied by the suppression of intratumoral survivin at the dose levels of 3 to 10 mg/kg/day (Nakahara et al., 2007). The mice were euthanized under ether anesthesia 72 h after infusion, after which the osmotic pump was removed. One mouse was used as the primary with the other as a backup. After the hair was removed with a pair of clippers, and the natural orifices such as the eyes, nose, mouth, ears, anus, and urogenital organs were sealed with 4% (w/v) carboxymethyl cellulose, the mice were frozen in dry ice/acetone. Afterwards, the bodies were embedded in 10% (w/v) carboxymethyl cellulose and refrozen, and then 30-μm-thick whole-body slices were made at −17°C using an autocryotome (PMV-450MP; Leica, Wetzlar, Germany). The slices were peeled off with adhesive tape, dried at −17°C, fixed to the imaging plate (SR-2025, Fuji Photo Film Co., Ltd., Tokyo, Japan), and exposed for a few days at room temperature. Radioluminograms were prepared by reading the radioactive images using a bioimaging analyzer (BAS-1800II; Fuji Photo Film Co., Ltd.) with a 65,536 (16 bit) grayscale and luminescence maximum detection efficiency of each radioactive area on the radioluminograms was calculated using TINA 2.0 software (Raytest, Straubenhardt, Germany) and expressed as PSL per square millimeter. The present dosing regimen (72-h constant subcutaneous infusion at a rate of 3 mg/kg/day) for [14C]YM155 is the same as that reported for nonradiolabeled YM155 (Nakahara et al., 2007). Preliminary results showed that the pumping rate is constant and that plasma radioactivity concentrations reached the steady state within 48 h after the start of infusion (data not shown). The concentration of radioactivity in the plasma 72 h into the constant subcutaneous infusion of [14C]YM155 was confirmed to be 22.3 ± 5.7 ng/ml (mean ± S.D. of three mice; unpublished data), which is comparable with that obtained for YM155 (approximately 20 ng/ml) in our previous study (Nakahara et al., 2007). Because YM155 remained virtually unmetabolized in vitro systems, such as hepatocytes, in mice and humans (Sohda et al., 2007). The radioactivity seen in the present experiments probably reflects the concentration of unchanged YM155.

In Vitro Binding of [14C]YM155 to PC-3 Xenograft Homogenate. Blank PC-3 xenografts were sampled from untreated nude mice bearing PC-3 tumors and homogenized on ice using a Potter-Elvehjem Teflon homogenizer. Phosphate buffer (0.01 M, with 0.14 M KCl, pH 7.2) was added to make final concentrations of 15 and 30% (w/v). The homogenate was spiked with [14C]YM155 (100 and 1000 ng/ml) and then incubated at 37°C for 10 and 60
[...]

concentration, either preheated at 37°C or on ice). A portion of the filtrate was transferred to an individual scintillation vial. The scintillator Hionic-Fluor (PerkinElmer Life and Analytical Sciences, Waltham, MA) was added to each vial, after which the radioactivity in each sample was counted in a liquid scintillation counter Tricarb-3100TR (Perkin-Elmer Life and Analytical Sciences). The counting efficiency was corrected using the external standard radiation source method. The unbound fraction of [14C]YM155 in the tumor tissue (i.e., 100% homogenate) was calculated using the following equation: $C_{b,100\%} / C_{u,100\%} = (100\% / XX\%) \times C_{u,XX\%} / C_{XX\%}$; unbound fraction in tumor tissue (%) = $|C_{b,100\%} / (C_{b,100\%} + C_{u,100\%})| \times 100 = (1 / (1 + C_{u,100\%} / C_{b,100\%})) \times 100$, where $C_{XX\%}$, unbound [14C]YM155 concentration in XX% homogenate ($= [14C]YM155$ concentration in filtrate); $C_{XX\%}$, bound [14C]YM155 concentration in XX% homogenate.

**In Vitro Protein Binding of YM155 in Mice.** Male BALB/c mouse plasma spiked with [14C]YM155 (10, 100, and 1000 ng/ml) was incubated at 37°C for 10 min and then transferred into an ultrafiltration tube (UltraTreat-10). After centrifugation at 1,870 g for 15 min at 37°C, each filtrate was transferred to a scintillation vial. In addition, the plasma samples not subjected to ultrafiltration were transferred to an individual scintillation vial. Scintillator was added to each vial, and the radioactivity in each sample was counted in a liquid scintillation counter as described above.

**In Vitro Blood-to-Plasma Concentration Ratios in Mice.** Male BALB/c mouse blood spiked with [14C]YM155 (5, 50, and 500 ng/ml) was incubated at 37°C for 10 min. After incubation, a portion of the blood sample was transferred into a scintillation vial. The hematocrit values were measured by using a portion of the spiked samples ($n = 2$). The remaining samples were centrifuged at approximately 1500g for 10 min at 37°C to obtain the plasma. A portion of the plasma sample was transferred into a scintillation vial. A liquid scintillator was added to the plasma samples. For blood samples, radioactivity was measured using the combustion method. The samples were placed on a combustion cone and dried in a drying oven at approximately 70°C for at least 8 h. The samples were combusted in a sample oxidizer (A0307 and A30701; PerkinElmer Life and Analytical Sciences) to prepare samples for the determination of the radioactivity. In the sample oxidizer, Carbo-SorbE and Permafluor E+ (PerkinElmer Life and Analytical Sciences) were used as carbon dioxide absorbent and liquid scintillator, respectively. In addition, before and after the combustion of the samples, the samples for the determination of recovery (referred to as recovery samples; $n = 3$) that contained the designated amount of radioactivity were combusted, and the radioactivity was measured. The radioactivity in the reference samples ($n = 3$) was determined using the direct measurement method. The recovery obtained in the sample oxidizer was calculated as the ratio of radioactivity in the recovery samples to that in the reference samples. The radioactivity was determined using the external standard radiation source method. The blood-to-plasma concentration ratio of [14C]YM155 was determined at concentrations ranging from 0.2 to 50 μM calculated using the following equations: blood-to-plasma concentration ratio = $C_{plasma} / C_{blood}$. Finally, the unbound [14C]YM155 cell-to-plasma concentration ratio ($C_{b,100\%} / C_{u,100\%}$) at both 37°C and on ice: at 37°C, the uptake at 37°C represents transporter-mediated uptake as well as passive diffusion and/or nonspecific binding. The Michaelis-Menten constant ($K_{m}$) and maximum uptake velocity ($V_{max}$) were estimated for each cell line using WinNonlin 4.1 (Pharsight, Mountain View, CA) by simultaneously fitting the following equations to the substrate concentration ([S]) versus uptake velocity (% of I) data: $V = V_{max} \times \left[ S / (K_{m} + S) \right] + P_{dif}$, where $V$ is the uptake velocity (% of I); $V_{max}$ is the maximum uptake velocity (% of I); $K_{m}$ is the Michaelis-Menten constant; $P_{dif}$ is the passive diffusion uptake velocity (% of I); $P_{dif}$ is the passive diffusion uptake velocity (% of I); and $P_{non}$ is the nonspecific binding uptake velocity (% of I).

**Results**

**In Vivo Distribution of [14C]YM155 in PC-3-Confined Nude Mice Using Whole Body Radioluminography.** The radioluminogram showing distribution of radioactivity 72 h after [14C]YM155 infusion is shown in Fig. 2. The highest levels of radioactivity were detected in the contents of the large (386.71 PSL/mm²) and small (28.20 PSL/mm²) intestines and were 348 and 25 times higher than that in the blood (1.11 PSL/mm²), respectively. Radioactivity in the tumor, kidney, and liver (16.36, 9.63, and 4.20 PSL/mm², respect...
intestinal contents. Kidney; 5, submaxillary gland; 6, skin; 7, small intestinal contents; and 8, large section of kidney (B), and section of center (C). 1, blood; 2, tumor; 3, liver; 4, mg/kg/day for 3 days, to a PC-3-xenografted nude mouse. Section of tumor (A), rest of the section (skin, bone, and muscles).

Tissues. In the section of slice containing tumor tissue (Fig. 2A), the intestinal contents, the highest radioactivity was observed in the tumor via bile after the distribution into the liver. Apart from the gastroin-systemic circulation, i.e., blood, was excreted into the intestinal tract suggested that the radioactivity absorbed subcutaneously into the rest of the section (skin, bone, and muscles).

In Vitro Binding of [14C]YM155 to the Tumor Tissue Homogenate. The in vitro unbound fraction of YM155 (%) in PC-3 tumor tissue ranged from 40.47 to 50.05%. The unbound fraction was independent of homogenate concentration (15 and 30%), YM155 concentration (100 and 1000 ng/ml), and incubation time (10 and 60 min).

In Vitro Unbound Fraction in Plasma and Blood-to-Plasma Concentration Ratio of [14C]YM155 in Mice. The in vitro unbound fraction of YM155 in mouse plasma ranged from 77.62 to 81.40% at the concentration range tested (Table 1), whereas plasma protein binding of YM155 was low. The in vitro blood-to-plasma concentration ratio and the blood cell-to-plasma concentration ratio were also low, indicating that very little YM155 was distributed into the blood cells (Table 1). Very low distribution into the blood cells and no concentration-dependent saturation suggested the lack of uptake transporter in blood cells.

Time Courses of the Uptake of [14C]YM155 into Cancer Cells. The time courses for the uptake of [14C]YM155 (1 μM) into PC-3, Calu-6, NCI-H358, A375, SK-MEL-5, RL, and Ramos cell lines are shown in Fig. 3. There was a markedly higher uptake at 37°C than on ice. For all cell lines, the uptake of [14C]YM155 increased linearly with the incubation time up to 5 min at 37°C.

Concentration-Dependent Uptake of [14C]YM155 into Cancer Cells. Eadie-Hofstee plots for the concentration dependence of the 5-min uptake of [14C]YM155 into PC-3, Calu-6, NCI-H358, A375, SK-MEL-5, RL, and Ramos cell lines suggested one saturable component (Fig. 4). The Kₘ, Vₘₐₓ, and Pₐₙₒ values are listed in Table 2. The Kₘ values were similar among these cell lines, ranging from 0.189 to 0.367 μM. This saturable concentration dependence as well as temperature dependence suggested the carrier-mediated uptake of [14C]YM155 into cancer cells.

Effects of Various Compounds on the Uptake of [14C]YM155 into Cancer Cells. The effects of various compounds, which shows different affinity to various organic cation and anion transporters, on the 5-min uptake of [14C]YM155 (0.1 μM, which is lower than Kₘ values) into PC-3, Calu-6, A375, RL, and Ramos cell lines are shown in Figs. 5 and 6. The inhibition patterns of the compounds were similar among the five tested cell lines. Of the compounds tested, only the cationic transporter substrates/inhibitors (tetraethylammonium [TEA], 1-methyl-4-phenylpyridinium [MPP], cinemidine, prazosin, corticosterone, verapamil, amantadine, procainamide, and N-methyl-N-nicotinamide [NMN]) inhibited the uptake of [14C]YM155 to a similar extent among the five cell lines. However, carnitine, an inhibitor of novel organic cation transporters OCTN1 (SLC22A4) and OCTN2 (SLC22A5), and OCT6 (Koepfle et al., 2007), did not affect the uptake at all (Fig. 5; Table 3). In addition, organic anion transporter substrates/inhibitors [probeneicd and p-aminophenolic acid for organic anion transporters (Burkhard and Burkhard, 2003); cyclosporine and taurocholate for organic anion transporter polypeptides (Fujiiwara et al., 2001; Kulak-Ublck et al., 2001; Shiota et al., 2005)] did not inhibit the uptake of [14C]YM155. The uric acid transporter inhibitor benz bromarone (Iwanaga et al., 2005) did not affect the uptake.

Subsequently, the concentration-dependent inhibitory effects of cationic transporter substrates/inhibitors tributylmethylammonium (TBMEA), MPP, cinemidine, prazosin, corticosterone quinidine, verapamil, amantadine, procainamide, and NMN on the 5-min uptake of [14C]YM155 (0.1 μM) into PC-3 were examined (figure not shown). The IC₅₀ values obtained are listed in Table 3. The IC₅₀ values of compounds inhibiting the human OCT1-, OCT2-, OCT3 (SLC22A3)-, OCTN1-, OCTN2-, and OCT6-mediated transport of prototypical substrates (instead of YM155) (Koepfle et al., 2003, 2007) obtained from the literature are also listed in Table 3. Each inhibitor shows different affinity to OCT1, OCT2, OCT3, OCTN1, OCTN2, and OCT6-mediated transport of prototypical substrates (instead of YM155) (Koepfle et al., 2003, 2007) obtained from the literature would be informative to identify the exact transporter involved in the uptake of YM155 into cancer cells. It was assumed that, for example, if YM155 had been transported by OCT1 in PC-3 cells, IC₅₀ values for the uptake of YM155 into PC-3 would have been consistent with those for

### Table 1

<table>
<thead>
<tr>
<th>[14C]YM155 Concentration</th>
<th>10 ng/ml (0.028 μM)</th>
<th>100 ng/ml (0.28 μM)</th>
<th>1000 ng/ml (2.8 μM)</th>
<th>5 ng/ml (0.014 μM)</th>
<th>50 ng/ml (0.14 μM)</th>
<th>500 ng/ml (1.4 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unbound fraction, %</td>
<td>77.62 (1.38)</td>
<td>79.14 (1.05)</td>
<td>81.40 (1.32)</td>
<td>0.59 (0.02)</td>
<td>0.59 (0.01)</td>
<td>0.58 (0.01)</td>
</tr>
<tr>
<td>Blood-to-plasma ratio</td>
<td>0.17 (0.004)</td>
<td>0.18 (0.03)</td>
<td>0.15 (0.02)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood cell-to-plasma ratio</td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
OCT1 for all inhibitors. However, the IC_{50} values of several compounds—TEA, tributylmethylammonium, MPP, cimetidine, corticosterone, and procainamide—on the uptake of [14C]YM155 into PC-3 were very different from the IC_{50} values for human OCT1-mediated transport of prototypical substrates from the literature. Therefore, a transporter(s) other than OCT1 may be involved in the uptake of [14C]YM155 into the cancer cells tested, although further investigation is needed. In addition, for OCT2 and OCT3, discrepancies were observed between the IC_{50} values for several compounds (MPP, cimetidine, corticosterone, and procainamide for OCT2 and OCT3; prazosin, amantadine, and NMN for OCT2) for the inhibition of [14C]YM155 uptake into PC-3 cells and those for the inhibition of transport of prototypical substrates by OCT2 and OCT3 in the literature (Table 3). This suggests that OCT2 and OCT3 do not play important roles in the uptake of [14C]YM155 into cancer cells.

**Extracellular pH-, Na\(^+\), and Cl\(^-\)-Dependent Uptake of [14C]YM155 into PC-3 Cells.** The effect of buffer pH on the uptake of [14C]YM155 into PC-3 cells at 37°C is shown in Fig. 7. When cells were placed on ice, uptake of [14C]YM155 on ice was not detectable. Uptake decreased as the pH lowered, although YM155 seems to maintain its positive charge over a wide range of pH values (unpublished data). The uptake of [14C]YM155 into PC-3 cells at 37°C when the Na\(^+\) in the buffer was replaced by Li\(^+\), choline\(^+\), or K\(^+\) is presented in Fig. 7. Again, uptake of [14C]YM155 on ice was not detectable. Li\(^+\) could be substituted for Na\(^+\), but when Na\(^+\) was replaced by K\(^+\) or choline\(^+\), the uptake decreased. When Na\(^+\) was replaced by K\(^+\), the
results suggested the voltage-dependent transport of YM155 into the PC-3 cells. When the Cl\textsuperscript{−} in the buffer was replaced by gluconate, a slight, but not marked, decrease in the uptake was observed (data not shown). The pH and Na\textsuperscript{+} dependencies (Fig. 7) were similar to those of organic cation transporters (Gruendemann et al., 1994; Kakehi et al., 2002). These results seem reasonable because YM155 has a cationic moiety in its structure (Fig. 1).

**Discussion**

YM155 is a new first-in-class molecular entity that suppresses survivin and shows potent antitumor activity in various types of cancer cells, including PC-3 cells, both in vitro and/or in vivo (Nakahara et al., 2007). YM155 showed high distribution into its pharmacologically effective site, tumor tissue, after administration to nude mice bearing PC-3 cells. This preferential distribution property is considered to result in antitumor activity without the induction of severe systemic toxicity in vivo (Nakahara et al., 2007). The in vitro 50% cell growth inhibition concentration for YM155 was 8.2 nM in PC-3 cells after treatment with YM155 for 48 h (Nakahara et al., 2007). At the pharmacologically effective dose, 3 mg/kg in vivo, the plasma concentration of YM155 was approximately 20 ng/ml (55 nM) at steady state during subcutaneous infusion of YM155 to nude mice.
bearing a PC-3 tumor (Nakahara et al., 2007). Given that the unbound fraction of YM155 in mouse plasma is approximately 80%, the unbound plasma concentration is estimated to be 44 nM. Therefore, the pharmacologically effective concentrations in vitro and in vivo are comparable. In addition, the metabolism of YM155 was very low (Sohda et al., 2007). Therefore, YM155 itself (rather than its metabolites, if any) could be considered to be the main pharmacologically active component, and the pharmacological target organ may be the tumor itself both in vivo and in vitro. The radioluminogram showed high levels of radioactivity in the tumor, kidney, and liver as well as the excreta (large intestinal contents and small intestinal contents) (Fig. 2). Only a trace of radioactivity was detected in the submaxillary gland and skin. These results suggested that, compared with most other organs, the greatest proportion of [14C]YM155 is distributed to the tumor, which is the target organ (the tumor-to-blood radioactivity ratio was 14.7). In general, high tissue distribution of a drug results from a high rate of binding of the drug to the cell surface and intracellular component, and/or carrier-mediated uptake into the cells. Taking the in vitro unbound fraction of YM155 in tumor tissue and plasma as well as the blood-to-plasma radioactivity ratio into account, the tumor-to-plasma concentration ratio based on the unbound concentration was estimated to be 4.3 to 5.6. For the tumor-to-plasma ratio of the total YM155 concentration (16.7–24.6) (Nakahara et al., 2007), the value was recalculated to be 9.5–13.9, based on the unbound concentration (unpublished data). This high ratio suggested that tumor-tissue binding alone could not fully explain the high distribution of YM155 in the tumor; therefore, carrier-mediated uptake of YM155 into the cancer cells was postulated.

### TABLE 2

Summary of kinetic parameters for the concentration-dependent uptake of [14C]YM155 into PC-3, Calu-6, NCI-H358, A375, SK-MEL-5, RL, and Ramos cells

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol/min/mg protein)</th>
<th>$P_{diff}$ (µl/min/mg protein)</th>
<th>$P_{diff}$ (µl/min/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-3</td>
<td>0.243 ± 0.015</td>
<td>14.93 ± 0.48</td>
<td>0.484 ± 0.033</td>
<td>0.117 ± 0.008</td>
</tr>
<tr>
<td>Calu-6</td>
<td>0.189 ± 0.020</td>
<td>4.38 ± 0.25</td>
<td>0.718 ± 0.036</td>
<td>0.432 ± 0.027</td>
</tr>
<tr>
<td>NCI-H358</td>
<td>0.254 ± 0.033</td>
<td>3.40 ± 0.25</td>
<td>0.711 ± 0.048</td>
<td>0.432 ± 0.027</td>
</tr>
<tr>
<td>A375</td>
<td>0.367 ± 0.042</td>
<td>6.87 ± 0.50</td>
<td>0.607 ± 0.047</td>
<td>0.432 ± 0.027</td>
</tr>
<tr>
<td>SK-MEL-5</td>
<td>0.359 ± 0.057</td>
<td>4.01 ± 0.41</td>
<td>0.484 ± 0.033</td>
<td>0.117 ± 0.008</td>
</tr>
<tr>
<td>RL</td>
<td>0.202 ± 0.008</td>
<td>2.56 ± 0.06</td>
<td>0.117 ± 0.008</td>
<td>0.432 ± 0.027</td>
</tr>
<tr>
<td>Ramos</td>
<td>0.283 ± 0.029</td>
<td>0.980 ± 0.059</td>
<td>0.0788 ± 0.0042</td>
<td>0.432 ± 0.027</td>
</tr>
</tbody>
</table>

**Fig. 5.** Effects of various compounds on [14C]YM155 (0.1 µM) uptake into PC-3, Calu-6, and A375 cells at 37°C for 5 min. Data are expressed as the mean (S.D.) of triplicate experiments. a, not detected.
Subsequently, a detailed characterization of the uptake of \[^{14}C\]YM155 into solid tumor and lymphoma cells was performed in vitro. The similarity of the results among the cell lines tested indicates similar uptake mechanism for the uptake of YM155 into solid tumor and lymphoma cells. The temperature- and saturable concentration-dependent uptake of \[^{14}C\]YM155 into solid tumor and lymphoma cell lines indicated the involvement of transporter(s). The \(K_m\) values were similar among the cell lines tested, suggesting similar uptake mechanism for the uptake of YM155 into solid tumor and lymphoma cells. It has been reported that water-soluble anticancer drugs, nucleoside analogs, and antifolates are taken up by drug transporters on the plasma membrane of the cancer cells (Huang and Sadée, 2006). Transporters of endogenous or nutrient substances such as folate, nucleic acid, and sugar have been studied extensively (Huang and

### TABLE 3

Summary of the IC\(_{50}\) values of various compounds against the uptake of \[^{14}C\]YM155 into PC-3 and comparison with the IC\(_{50}\) values from the literature for human OCT1, OCT2, OCT3, OCTN1, OCTN2, and OCT6

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC(_{50}) (μM)</th>
<th>IC(_{50}) (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OCT1</td>
<td>OCT2</td>
</tr>
<tr>
<td>D-Carnitine</td>
<td>&gt;1000</td>
<td></td>
</tr>
<tr>
<td>L-Carnitine</td>
<td>&gt;1000</td>
<td>12,400</td>
</tr>
<tr>
<td>TEA</td>
<td>&gt;1000</td>
<td>158; 173</td>
</tr>
<tr>
<td>TBMEA</td>
<td>458</td>
<td>66</td>
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<tr>
<td>MPP</td>
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<td>12</td>
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<td>Cimetidine</td>
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<td>Prazosin</td>
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</tr>
<tr>
<td>Corticosterone</td>
<td>&gt;100</td>
<td>7; 22</td>
</tr>
<tr>
<td>Quinidine</td>
<td>3.62</td>
<td>18</td>
</tr>
<tr>
<td>Verapamil</td>
<td>4.38</td>
<td>2.9</td>
</tr>
<tr>
<td>Amantadine</td>
<td>993</td>
<td>236</td>
</tr>
<tr>
<td>Procainamide</td>
<td>987</td>
<td>74; 107</td>
</tr>
<tr>
<td>NMN</td>
<td>4209</td>
<td>7700</td>
</tr>
</tbody>
</table>

\(a\) Values are cited from the review articles by Koepsell et al. (2003, 2007).

\(b\) Based on the results in Fig. 5.

\(c\) \(K_m\) values.

**Fig. 6.** Effects of various compounds on \[^{14}C\]YM155 (0.1 μM) uptake into RL and Ramos cells at 37°C for 5 min. Data are expressed as the mean (S.D.) of triplicate experiments. a, not detected.
an inhibitor of OCT1, OCT2, and OCT6, did not inhibit the uptake of YM155 (Fig. 5; Table 3). As well as the involvement of OCTs mentioned above, other of those cation transporter, organic anion transporters that also transport organic cations, endogenous cationic substrate transporters, and others should be investigated in future studies.

For the distribution of YM155 into normal tissues instead of tumor, the high levels of radioactivity detected in the contents of the large and small intestines (Fig. 2) were well explained by the biliary excretion of radioactivity after high distribution into the liver. Biliary excretion is a major excretion route of YM155 in rats and dogs (unpublished data). Because YM155 is transported by human OCT1 and because OCT1 is highly expressed in mouse liver (Mooslehner and Allen, 1999; Alnouti et al., 2006), it is likely YM155 is taken up into the hepatocytes by OCT1 in mice. Because OCT1 is also located on the basolateral membrane of enterocytes in mice (Koepsell et al., 2007), the distribution of YM155 into these enterocytes from the blood is also probable. In Oct1-knockout mice, the concentrations of TEA in the liver and small intestine decreased, as did the biliary excretion of TEA (Koepsell et al., 2007). It is likely that more than one transporter is involved in the pharmacokinetics of YM155. OCT1 is involved in the distribution of YM155 into the liver, another (or more) is involved in uptake into cancer cells, and still others for uptake into other tissues.

In conclusion, YM155 showed high distribution to tumor tissues after continuous administration to nude mice bearing PC-3 tumors. The in vitro experiments suggested that YM155 was taken up into various cancer cells in a carrier-mediated manner. This process occurred with similar affinity among the cancer cell lines tested: prostate cancer, lung cancer, melanoma, and non-Hodgkin’s lymphoma. A drug transporter(s) for organic cations may contribute to this process. Further investigation is needed to identify the transporter for possible optimization of cancer chemotherapy using YM155.

References


its functional comparison with three other OATPs of human liver. Gastroenterology 120:525–533.


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