Modulation of the Tumor Disposition of Vinca Alkaloids by PSC 833 In Vitro and In Vivo

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ABSTRACT

PSC 833, a nonimmunosuppressive cyclosporin, is able to inhibit the efflux of antitumor drugs mediated by P-glycoprotein (P-gp). The purpose of the present study is to compare the effect of PSC 833 on the tumor disposition of [3H]vincristine ([3H]VCR) and [3H]vinblastine ([3H]VBL) in vitro and in vivo experiments from a pharmacokinetic point of view. In in vitro experiments, the effect of PSC 833 was investigated on the cellular uptake of [3H]VCR and [3H]VBL by HCT-15 and COLO 205, human colorectal tumor cell lines with extensive and minimal expression of P-gp, respectively. PSC 833 (2 μM) increased the cellular uptake of [3H]VCR and [3H]VBL by HCT-15 cells, but not that by COLO 205 cells, 8- and 6-fold, respectively, without affecting the initial influx rates. In addition, 2 μM PSC 833 reduced the efflux of [3H]VCR from HCT-15 cells to a level comparable with that from COLO 205 cells. Furthermore, the effect of PSC 833 on the tumor disposition of intravenously administered [3H]VCR and [3H]VBL was studied in tumor inoculated mice. Infusion of PSC 833 (10 μg/hr/mouse) increased the HCT-15 tumor disposition of [3H]VBL to a level comparable with that observed in vitro. These findings demonstrate that PSC 833 enhances the tumor disposition of vinca alkaloids by inhibition of P-gp-mediated efflux not only in vitro but also in vivo in a solid tumor model.

MDR is one of the major obstacles to successful tumor chemotherapy. The most important factor involved in the development of MDR is overexpression of P-gp, an efflux pump for hydrophobic antitumor drugs, encoded by the MDR1 gene (Pastan and Gottesman, 1987). One of the most convincing methods to overcome the MDR caused by P-gp is to use a monoclonal antibody. Iwahashi et al. (1993) examined the disposition of MRK 16, a monoclonal antibody against P-gp (Hamada and Tsuruo, 1986), in tumor-bearing mice and found selective accumulation of this antibody to the tumor extensively expressing the antigen. We also demonstrated the kinetic basis for the selective in vivo accumulation of this antibody to the tumor by means of an in vitro binding study (Mano et al., 1997).

The other method of overcoming the MDR mediated by P-gp is to use ligands with the ability to inhibit P-gp function. Until now, a number of compounds, including verapamil and CsA, have been found to inhibit P-gp function both in vitro and in vivo (Lum et al., 1993). However, it is difficult to use these compounds as P-gp modifiers due to their pharmacological actions. To overcome this problem, PSC 833, a compound with no immunosuppressive action, was synthesized as a CsA analog. PSC 833 is one of the most potent agents as far as MDR reversal is concerned (Keller et al., 1992a, 1992b; Watanabe et al., 1995b) and therefore has been introduced into clinical use (Raderer and Scheithauer, 1993).

Preclinical studies to discover whether PSC 833 can enhance the tumor disposition of antitumor drugs in vivo, just as it does in vitro, are of obvious clinical importance. Preclinical data on the combination of MDR modulators with antitumor drugs in vivo are limited and what there are have been confined to the murine leukemia model (Cros et al., 1992; Colombo and Gonzalez, 1996). One of the clearest pieces of evidence to support the importance of PSC 833 is the finding that the concomitant administration of PSC 833, CsA or verapamil with VCR or ADR increased the survival time of P388/ADR-bearing mice (Watanabe et al., 1995b). However, a report has appeared that demonstrates that pretreatment with PSC 833 is ineffective in the treatment of P388/ADR leukemia with ADR in vivo (Colombo and Gonzalez, 1996). Furthermore, the information on solid tumors is much more limited and controversial (Horton et al., 1989; Watanabe et al., 1995b). One of the reasons for such contradictory results

ABBREVIATIONS: MDR, multidrug resistance; P-gp, P-glycoprotein; VCR, vincristine; VBL, vinblastine; ADR, adriamycin; CsA, cyclosporin A; Kp, tissue-to-plasma concentration ratio; KZI, Konzentrat Zuer Infusion; HPLC, high-performance liquid chromatography
may be ascribed to a loss of acquired P-gp activity after inoculating the nude mice (Broxterman et al., 1995). Therefore, it is necessary to use cell lines that stably express P-gp to evaluate the activity of PSC 833 in any in vivo experiments.

In addition, it is reported that P-gp is expressed also in many somatic cells such as hepatocytes and renal tubular epithelial cells (Cordon-Cardo et al., 1990) and has the functional significance to determine the disposition of its substrates e.g., we clarified the role of P-gp located on the bile canalicular membrane in the biliary excretion of VCR by demonstrating the inhibitory effect of verapamil along with the increased excretion in rats whose P-gp expression was induced by phenothiazine treatment (Watanabe et al., 1992, 1995a). According to the previous observations from this and other laboratories, it is possible that the disposition of P-gp substrates within the body may be altered by concomitantly administered PSC 833 (Lum et al., 1993; Kusuhara et al., 1997).

Based on such background information, we characterized here the effect of PSC 833 on the disposition of vinca alkaloids in an in vivo tumor-bearing mouse model in relation to the in vitro uptake. For the present analysis, we used HCT-15, a human colorectal tumor cell line that extensively expresses P-gp constitutively (Iwashashi et al., 1993); COLO 205, another human colorectal tumor cell line with minimal P-gp expression (Iwashashi et al., 1993), was used as a control. The expression of P-gp in HCT-15, but not in COLO 205, has been confirmed by examining the binding of 125I-MRK-16, monoclonal antibody against P-gp, in vitro and in tumor inoculated nude mice in vivo (Mano et al., 1997). As antitumor drugs, we used two vinca alkaloids (VCR and VBL) with different lipophilicity.

**Experimental Procedures**

**Materials.** [3H]VCR (2–10 Ci/mmol) and [3H]VBL (5–25 Ci/mmol) were purchased from Amersham (Buckinghamshire, England) and Moraveck Biochemicals (Brea, CA), respectively. VCR and VBL were supplied by Novartis Pharma (Basel, Switzerland). All other chemicals and reagents were commercial products of analytical grade.

**Animals and tumor cells.** HCT-15 and COLO 205 from American Type Culture Collection (Rockville, MD) were cultured in RPMI 1640 supplemented with 10% fetal calf serum in a humidified atmosphere with 5% CO₂ at 37°C. Six week-old female BALB/c nu/nu mice weighing 20 to 22 g were purchased from Japan Laboratory Animals (Tokyo, Japan). PSC 833 was supplied by Novartis Pharma (Basel, Switzerland). All other chemicals and reagents were commercial products of analytical grade.

**Uptake study.** Cells (2 × 10⁶) were seeded on 24-well plate 24 hr before the experiments. The uptake of [3H]VCR (0.05 μM) and [3H]VBL (0.01 μM) by the cultured cells was examined at 37°C using uptake medium consisting of RPMI 1640 with 10% fetal calf serum pH adjusted to 7.4 by N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (20 mM). A warm plate was used to adjust the temperature to 37°C. At specified times, the cells were washed three times with ice-cold phosphate-buffered saline and then solubilized with 250 μl of 1 N NaOH. The radioactivity was counted in a liquid scintillation counter (model LS6000LI; Beckman, Galway, Ireland).

To investigate the effect of PSC 833 on the influx process, we determined the initial uptake rate of the vinca alkaloids by each cell line from linear regression of the profiles for the initial uptake; 10, 20 and 60 sec data for [3H]VCR and 10 and 20 sec data for [3H]VBL were obtained in quadruplicate and analyzed. Because our data indicated that there was linearity in the initial uptake of vinca alkaloids, at least, up to the last time point used for the analysis, the initial uptake velocity can be determined correctly based on this method.

In in vitro experiments, PSC 833, dissolved in ethanol, was added to the medium. The final concentration of ethanol was 0.1% throughout the in vitro experiment. The control experiments were also performed in the presence of 0.1% ethanol.

To examine the cellular efflux of [3H]VCR, cells were preloaded with [3H]VCR (0.05 μM) for 2 hr. Then they were washed with ice-cold phosphate-buffered saline and the previously described medium free from [3H]VCR was added to examine the efflux. At the specified times, the cells were washed, and then the radioactivity associated with the cells was determined as described previously.

**In vivo study.** Nude mice were inoculated subcutaneously with 3 × 10⁶ and 5 × 10⁶ HCT-15 and COLO 205 cells suspended in 0.2 ml saline into the right and left peritoneal flanks, respectively. After 11 days, PSC 833 dissolved in a vehicle, Konsentrant Zuer Infusion (KZI) (cremophor/ethanol = 65/35 v/v) was infused intraperitoneally at a rate of 2, 10 and 20 μg/mouse via an osmotic pump (model ALZET 1007D; ALZA Scientific Product, Palo Alto, CA) until the end of the experiments. As a control, KZI or saline was infused in the same manner, at a rate of 0.5 μg/mouse. Twenty-four hours after the initiation of PSC 833 infusion, [3H]VBL (10 μCi, 2 mg/kg) or [3H]VCR (10 μCi, 0.5 mg/kg) was injected through the tail vein. Mice were killed at 5 and 24 hr after administration of [3H]VCR and [3H]VBL, respectively. The tumor volume measured just after death was 250 to 500 mg for both HCT-15 and COLO 205 tumors. Before death, blood was collected from the heart, and then plasma was obtained by centrifugation. Tissues (HCT-15 tumor, COLO 205 tumor, brain, liver, kidney, intestine, adrenal, spleen and lung) were excised to determine the amount of [3H]VBL and [3H]VCR using the following method.

**High-performance liquid chromatography analysis.** The analysis of [3H]VCR and [3H]VBL was accomplished by HPLC as described previously (Belle et al., 1992) after extraction with diethyl ether (Tellengen et al., 1993) with slight modification. Briefly, tissues were homogenized with 2 ml of ice-cold saline with a Polytron homogenizer (Ulta-Turrax T25, IKA Labortechnik, Staufen i. Br., Germany). Then, 10 μg of unlabeled VCR or VBL was added to 100 μl of plasma and to 2 ml of each homogenates as an internal standard. The specimens were mixed for 10 sec and placed for 30 min on the ice for equilibrium. Vinca alkaloids associated with the specimens were extracted twice with 2 ml diethyl ether. Diethyl ether layer was collected and evaporated under a gentle stream of nitrogen at 37°C. The residue was reconstituted in 200 μl of eluent and 100 μl of reconstituent was injected onto the HPLC column. The eluent fraction corresponding to the VBL and VCR peaks was collected to determine the radioactivity. The radioactivity was corrected by the recovery calculated using unlabeled internal standard. In most cases, several radioactive peaks were observed in in vivo specimens. For example, only 25% to 35% of radioactivities in plasma specimens at 5 hr after i.v. administration represents intact [3H]VCR. Other peaks should represent the metabolites and/or degraded materials.

The HPLC system consisted of a pump (model L-6200; Hitachi, Tokyo, Japan), an analytical column (YMC-PACK CN A502, 5 μm CN-bonded phase, 150 × 4.6 mm, Yamamura Chemical Laboratories, Kyoto, Japan) with a guard column (C-KGC-524C-3, 5 μm CN-bonded phase, Yamamura Chemical Laboratories), an autosampler (model 851-AS; Tusco, Tokyo, Japan), a spectrophotometer detector (model L-4200; Hitachi) and a fraction collector (model L-5200; Hitachi). The wavelength for the analysis was 210 nm. The mobile phase consisted of acetonitrile, H₂O and phosphoric acid. The ratio of these solvent was 20:80:0.16 for VCR and 23:77:0.15 for VBL. The flow rate was set at 0.5 ml/min.

**Statistical method.** The results are shown as mean ± S.E. of the number of determinations. Either Student’s t test or ANOVA fol-
Results

Effect of PSC 833 on uptake of [3H]VCR and [3H]VBL. The uptake of [3H]VCR and [3H]VBL by COLO 205 cells increased with time and was ~10- and 6-fold greater than that by HCT-15 cells, respectively (fig. 1). 2 μM PSC 833 increased the net uptake of vinca alkaloids by HCT-15 cells to a level comparable with that by COLO 205 cells (fig. 1). In contrast, the net uptake of vinca alkaloids by COLO 205 cells was not affected by 2 μM PSC 833 (fig. 1). The initial uptake rate of both vinca alkaloids by COLO 205 cells was slightly greater than that by HCT-15 cells (table 1). In addition, 2 μM PSC 833 did not affect the initial uptake rate of vinca alkaloids by both cell lines (table 1).

We examined the effect of PSC 833 on the efflux of [3H]VCR from HCT-15 and COLO 205 cells; 2 μM PSC 833 did not affect efflux of [3H]VCR from COLO 205 cells (fig. 2). The efflux rate of [3H]VCR from HCT-15 cells was approximately twice that from COLO 205 cells and 2 μM PSC 833 reduced the efflux from HCT-15 cells to a level comparable with that by COLO 205 cells (fig. 2).

A concentration-dependent effect of PSC 833 on the accumulation of [3H]VCR and [3H]VBL was also observed (fig. 3). In addition, we examined the saturable uptake of the vinca alkaloids in the presence and absence of PSC 833. In the absence of PSC 833, no significant increase in the cell-to-medium ratio was observed in HCT-15 cells, whereas the cell-to-medium ratio of COLO 205 cells was reduced in a dose-dependent manner (fig. 4). PSC 833 (2 μM) increased the uptake of vinca alkaloids by HCT-15 cells to a level comparable with that by COLO 205 cells (fig. 4).

Effect of PSC 833 infusion on the tumor disposition of vinca alkaloids. The effect of PSC 833 on the tumor disposition of vinca alkaloids was also examined in the in vivo experiments. Infusion of KZI had no effect on the tumor disposition of vinca alkaloids (figs. 5 and 6). Administration of PSC 833 significantly increased the tumor concentration of vinca alkaloids in both COLO 205 and HCT-15 tumors (figs. 5 and 6). In PSC 833-treated mice, the disposition of vinca alkaloids to HCT-15 tumors was increased to a level comparable with that to COLO 205 tumors (figs. 5 and 6). In PSC 833-treated mice, the disposition of vinca alkaloids (figs. 5 and 6). In PSC 833-treated mice, the disposition of vinca alkaloids (figs. 5 and 6). In PSC 833-treated mice, the disposition of vinca alkaloids (figs. 5 and 6).

Effect of PSC 833 on the efflux of [3H]VCR from HCT-15 and COLO 205 cells. Cells were preincubated for 30 min at 37°C in the absence and presence of PSC 833 (2 μM) in the medium supplemented with 10% fetal calf serum. PSC 833, dissolved in ethanol, was added to the medium. The final concentration of ethanol was 0.1% throughout the in vitro experiment. Control experiments were also performed in the presence of 0.1% ethanol. Initial uptake rates were calculated from the linear regression of the initial uptake. Data are mean ± calculated S.E.

![Fig. 1. Effect of PSC 833 on the uptake of [3H]VCR (A) and [3H]VBL (B) by HCT-15 and COLO 205 cells. Cells were preincubated for 30 min at 37°C in the medium with and without PSC 833 (2 μM). Accumulation of [3H]VCR (0.05 μM) and [3H]VBL (0.01 μM) was examined as a function of time in the presence and absence of PSC 833 (2 μM). PSC 833, dissolved in ethanol, was added to the medium. The final concentration of ethanol was 0.1% throughout the in vitro experiment. Control experiments were also performed in the presence of 0.1% ethanol. The result is given as the volume of distribution (μg/mg protein) defined as the amount of ligand associated with the cells (μmol/mg protein) divided by the ligand concentration in the medium (μmol/l). Data are mean ± S.E. of four independent experiments. ○, HCT-15 control; ●, HCT-15 with 2 μM PSC 833; □, COLO 205 control; ■COLO 205 with 2 μM PSC 833.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PSC 833 (2 μM)</th>
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<tr>
<td>[3H]VCR</td>
<td></td>
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<tr>
<td>HCT-15</td>
<td>2.92 ± 0.87</td>
<td>2.93 ± 0.87</td>
</tr>
<tr>
<td>COLO 205</td>
<td>3.54 ± 2.25</td>
<td>5.60 ± 1.52</td>
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<tr>
<td>[3H]VBL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT-15</td>
<td>12.3 ± 5.4</td>
<td>19.2 ± 4.2</td>
</tr>
<tr>
<td>COLO 205</td>
<td>37.0 ± 6.8a</td>
<td>35.1 ± 13.3</td>
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* P < 0.05, significantly different from HCT-15 cells by Student’s t test.

Fig. 2. Effect of PSC 833 on the efflux of [3H]VCR from HCT-15 and COLO 205 cells. Cells were preloaded with [3H]VCR for 2 hr to examine the efflux in the presence and absence of PSC 833 (2 μM). To examine the effect of PSC 833, 2 μM of PSC 833 was added to the medium 30 min prior to the initiation of the efflux experiment. PSC 833, dissolved in ethanol, was added to the medium. The final concentration of ethanol was 0.1% throughout the in vitro experiment. Control experiments were also performed in the presence of 0.1% ethanol. Data are mean ± S.E. of four independent experiments. ○, HCT-15 control; ●, HCT-15 with 2 μM PSC 833; □, COLO 205 control; ■COLO 205 with 2 μM PSC 833.
of [3H]VBL (2 mg/kg). Tissue concentration was determined at 24 hr after saline were infused with the osmotic infusion pump 24 hr before injection. PSC 833 in KZI vehicle, KZI and 205 cells were inoculated to the left and right peritoneal flanks, respectively, 11 days before the experiment. PSC 833 inhibits the efflux of vinca alkaloids mediated by P-gp, and they also support the possibility that HCT-15 and COLO 205 cell lines can be used as an experimental model to evaluate in vitro modulation of P-gp function from HCT-15 and COLO 205 cells. Reduced the efflux of VCR from HCT-15 cells to a level comparable with that from COLO 205 cells, whereas the efflux of VCR from COLO 205 was unaffected by PSC 833. In addition, no significant effect of PSC 833 on the initial uptake of vinca alkaloids by both cell lines was observed (table 1). The half-maximum enhancement of the cellular uptake of vinca alkaloids by HCT-15 cells produced by PSC 833 was achieved at a concentration of 0.05 μM in the medium (fig. 3), which is comparable with previously reported values (Ludescher et al., 1995a; Watanabe et al., 1995b). Collectively, the in vitro data are consistent with the previous hypothesis that PSC 833 inhibits the efflux of vinca alkaloids mediated by P-gp, and they also support the possibility that HCT-15 and COLO 205 cells may be used as an experimental model to evaluate in vitro modulation of P-gp function from in vivo data. Because PSC 833 did not alter the cellular accumulation of vinca alkaloids in COLO 205 cells, PSC 833 may not affect the binding of these antitumor drugs to tubulin, the predominant factor determining vinca alkaloid disposition (Wierzba et al., 1987, 1988). This result also suggests that PSC 833 may not inhibit the cytotoxicity of vinca alkaloids per se.

In addition, the concentration-dependent uptake of vinca alkaloids was studied. Addition of 2 μM PSC 833 to the medium increased the uptake profiles of both vinca alkaloids by HCT-15 cells to a level comparable with that by COLO 205 cells (fig. 4). Figure 4 further indicates that the accumulation of vinca alkaloids by HCT-15 cells in the presence of PSC 833 and by COLO 205 cells exhibits saturation, presumably due to the saturation of the uptake and/or intracellular binding.

Discussion

In the present study, we evaluated the effect of PSC 833 on P-gp function both in vitro and in vivo. Increased cellular accumulation of VCR and VBL by HCT-15 cells, but not by COLO 205 cells, in the presence of PSC 833 (fig. 1) is attributed to the difference in the level of P-gp expression between the two cell lines (Iwahashi et al., 1993; Mano et al., 1997). These results were further supported by the efflux experiments from HCT-15 and COLO 205 cells (fig. 2); PSC 833
The linear accumulation of vinca alkaloids by HCT-15 cells in the absence of PSC 833 over the concentration range examined (fig. 4) may be accounted for by saturation of the uptake and/or intracellular binding. In clinical trials, however, a maximum effect of PSC 833 on the tumor disposition may be observed, because the therapeutic plasma unbound concentration of VCR would be ~0.05 μM (Nelson, 1982; Reynolds, 1996). For VBL, the extensive plasma protein binding makes such prediction difficult.

In the in vivo experiments, we found that the infusion of PSC 833 markedly increased the disposition of vinca alkaloids to HCT-15 tumor up to a level comparable with that to COLO 205 tumor (figs. 5 and 6). A maximum effect of PSC 833 was observed even at an infusion rate of 2 μg/hr/mouse (fig. 5). Our preliminary experiments indicated that the plasma concentration of PSC 833, measured with HPLC (Song et al., 1998), is 0.2, 1 and 2 μM at the infusion rates of 2, 10 and 20 μg/hr/mouse, respectively. In the in vitro cellular uptake studies, the ratio of the uptake by HCT-15 cells to that by COLO 205 cells for VCR and VBL was 0.09 and 0.16, respectively, in the absence of PSC 833 and this increased to 0.76 for both compounds in the presence of 2 μM PSC 833 (fig. 1). In the in vivo experiments, the corresponding ratios were 0.24 and 0.29 for the controls and 0.60 and 0.64 for the PSC 833-infused groups (10 μg/hr/mouse), respectively (figs. 5 and 6). Thus, there was good agreement in the in vitro and in vivo ratios for both vinca alkaloids.

Infusion of PSC 833 increased VCR and VBL association not only in HCT-15 tumor but also in COLO 205 tumor (figs. 5 and 6), although COLO 205 in vitro showed no PSC 833-dependent cellular uptake of these antitumor drugs (figs. 1 and 2). It was also found that plasma concentration of VCR in the PSC 833 treated group was also increased and, therefore, the tumor-to-plasma concentration ratio of VCR in COLO 205 was not affected by PSC 833 (table 2). Collectively, the increased tumor association of vinca alkaloids by PSC 833 in COLO 205 could be explained by the increase in plasma concentration, whereas in HCT-15 can be explained by considering the inhibition of P-gp-mediated efflux.

Although some reports have been published that suggest that cremophor, a major component of KZI, also inhibits P-gp function (Jette et al., 1995; Cufer et al., 1995; Spoelstra et al., 1991) at a concentration of 0.1 mg/ml in vitro, its in vivo effect is controversial; Ellis et al. (1996) examined the effect of cremophor on the elimination of etoposide in isolated perfused liver. They found an inhibitory effect of cremophor on the biliary excretion of etoposide if 80 mg of this ligand was added to the reservoir (100 ml) in the perfused liver. In contrast, i.v. or i.p. administration of cremophor at a dose of 1.44 g/kg failed to enhance the antitumor activity of Adriamycin in VCR-resistant P388 leukemia-bearing mice (Watanabe et al., 1996). In the present study, no significant effect of cremophor (3.3 μg/hr/mouse) on the tumor disposition of vinca alkaloids was observed (figs. 5 and 6).

PSC 833 also altered the disposition of VCR in mice (table 2). It has been reported that the disposition of P-gp substrates is modified by the concomitant administration of PSC 833 (Didier and Loof, 1996; Boote et al., 1996; Colombo and Gonzalez, 1996). For example, the plasma concentration profiles of Adriamycin in mice and etoposide in humans are altered by PSC 833 (Boote et al., 1996; Colombo and Gonzalez, 1996). Increased plasma concentrations of VCR (table 2) may result from modification of the biliary excretion mediated by P-gp, one of the predominant pathways for the excretion of VCR from the body (Watanabe et al., 1995a, 1995b). Regardless of the expression level of P-gp, the Kp of most of the normal tissues was not affected significantly by infusion of PSC 833. Our observation is consistent with previous data showing that the Kp value of several P-gp substrates, such as VBL, digoxin, CsA, dexamethasone and ivermectin, was not

### Table 2: Plasma concentration and tissue to plasma concentration ratio of [3H]VCR

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Plasma concentration (nM)</th>
<th>Tissue to plasma concentration ratio</th>
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<tbody>
<tr>
<td>HCT 15</td>
<td>2.99 ± 0.13, 3.32 ± 0.17</td>
<td>5.50 ± 0.29a,b</td>
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<tr>
<td>COLO 205</td>
<td>8.23 ± 0.20</td>
<td>6.92 ± 0.86</td>
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<tr>
<td>Liver</td>
<td>18.7 ± 0.9</td>
<td>27.7 ± 8.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>110 ± 5</td>
<td>89.0 ± 6.1</td>
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<tr>
<td>Adrenal</td>
<td>36.9 ± 3.7</td>
<td>30.5 ± 2.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>27.3 ± 3.1</td>
<td>23.2 ± 2.3</td>
</tr>
<tr>
<td>Intestine</td>
<td>32.0 ± 3.4</td>
<td>27.7 ± 0.7</td>
</tr>
<tr>
<td>Muscle</td>
<td>32.3 ± 1.7</td>
<td>28.2 ± 1.8</td>
</tr>
<tr>
<td>Heart</td>
<td>38.2 ± 2.5</td>
<td>28.1 ± 2.3</td>
</tr>
<tr>
<td>Lung</td>
<td>93.0 ± 4.5</td>
<td>68.4 ± 16.3</td>
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<tr>
<td>Brain</td>
<td>n.d.</td>
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<td>Brain</td>
<td>n.d.</td>
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n.d., not detectable.

* P < .05, ** P < .01, significantly different from the control (saline administered) group by ANOVA followed by Fisher’s t test.

** P < .05, ** P < .01, significantly different from the control (KZI administered) group by ANOVA followed by Fisher’s t test.

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**Fig. 6.** Tumor uptake of [3H]VCR in Balb/c nu/nu mice xenografted with HCT-15 and COLO 205 cells. 3 × 10^6 and 5 × 10^6 of HCT-15 and COLO 205 cells were inoculated to the left and right peritoneal flanks, respectively, 11 days before the experiment. PSC 833 in KZI vehicle, KZI and saline were given by intraperitoneal infusion using an osmotic infusion pump 24 hr before injection of [3H]VCR (0.5 mg/kg). Tissue concentration was determined at 5 hr after injection of [3H]VCR into the tail vein. Data are mean ± S.E. from three to four independent experiments. Statistical significance was determined against each saline infused group. * P < .05, ** P < .01 by ANOVA followed by Fisher’s t test.
significantly different between normal and mdr1a knockout mice in most of the normal tresses, except the brain (Schinkel et al., 1994, 1995), where the isotope count associated with the brain was below the limit of detection in the present study (table 2). It is plausible that penetration of VCR into the brain may be enhanced by PSC 833 treatment, since P-gp on the cerebral endothelial cells plays an important role in determining the Kp values of the previously described P-gp substrates in the brain. Although Lemaire et al. (1996) found that the penetration of VCR through the rat blood-brain barrier is increased by administration of PSC 833, by determining the VCR content associated with the brain parenchyma after washing out the blood remaining in the cerebral vascular system, they were unable to demonstrate that PSC 833 (10 mg/kg i.v.) could increase the Kp value of VCR in the brain 2 hr after administration.

In conclusion, treatment with PSC 833 resulted in an increased accumulation of vinca alkaloids in HCT-15 cells in vitro and in vivo. These results provide the basis for the pharmacological effect of PSC 833 in restoring the drug sensitivity of P-gp-positive tumors in vivo. In addition, we demonstrated that the in vivo effect of PSC 833 in increasing the tumor concentration of vinca alkaloids can be predicted from in vitro experiments. Changes in the pharmacokinetic of vinca alkaloids caused by coadministration of PSC 833 should also be considered in developing a safe and efficient tumor treatment.

References

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