Circumvention of Multidrug Resistance by a Newly Synthesized Quinoline Derivative, MS-073

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ABSTRACT

Newly synthesized quinoline derivatives were investigated for their efficacy to reverse multidrug resistance (MDR). In this study, one of the most effective quinoline derivatives, MS-073, was compared with verapamil with regard to its ability to overcome MDR in vitro and in vivo. MS-073 at 0.1 μM almost completely reversed in vitro resistance to vincristine (VCR) in VCR-resistant P388 cells. The compound also reversed in vitro VCR, adriamycin (ADM), etoposide, and actinomycin D resistance in ADM-resistant human myelogenous leukemia K562 (K562/ADM) cells, ADM-resistant human ovarian carcinoma A2780 cells, and colchicine-resistant human KB cells. MS-073 administered i.p. daily for 5 days with VCR enhanced the chemotherapeutic effect of VCR in ADM-resistant P388-bearing mice. Increases in life span of 19-50% were obtained by the combination of 10 μg/kg of VCR with 3-100 mg/kg of MS-073, as compared to the control. The ability of MS-073 to reverse MDR was remarkably higher, especially at low MS-073 doses, than that of verapamil, both in vitro and in vivo.

MS-073 enhanced accumulation of [3H]VCR in K562/ADM cells. Photolabeling of P-glycoprotein with 200 nM [3H]azidopine in K562/ADM plasma membranes was completely inhibited by 10 μM MS-073, indicating that MS-073 reverses MDR by competitively inhibiting drug binding to P-glycoprotein.

INTRODUCTION

One of the major problems in cancer chemotherapy is the development of drug resistance during treatment. When tumor cells acquire resistance to naturally occurring antitumor agents such as Vinca alkaloids or anthracyclines, they generally show cross-resistance to other antitumor agents having different structures and different modes of action (1-3). A major mechanism of this type of resistance is attributed to the reduced accumulation of antitumor agents in resistant cells (4-7). The P-170 glycoprotein is considered to cause the active efflux of antitumor agents from the resistant cells (8-12).

It is well known that verapamil and other calcium channel blockers inhibit the efflux of antitumor agents, thus overcoming MDR (13-18). Preliminary clinical studies have been carried out combining calcium channel blockers with chemotherapy. The combination chemotherapy is potentially useful against refractory acute lymphocytic leukemia of children, various advanced solid tumors, and small cell lung cancer but induces side effects such as reversible hypotension and arrhythmias (19-21). Therefore, more effective agents with fewer side effects than the present calcium channel blockers are needed.

We synthesized a new compound with potent ability to overcome MDR, while having relatively low host toxicity. In this study, the new compound, MS-073, was compared with verapamil in regard to its ability to overcome MDR both in vitro and in vivo. We found that MS-073 was more effective than verapamil in the interaction with P-glycoprotein and in reversing drug resistance.

MATERIALS AND METHODS

Drugs. VCR was supplied by Shionogi and Co., Ltd., Osaka, Japan. VP-16 was provided by Dr. M. Ogawa of this center. ADM and ACD were obtained from commercial sources. [3H]VCR (5.6 Ci/mmol) and [3H]azidopine (50 Ci/mmol) were purchased from Amersham Japan Ltd., Tokyo, Japan. Verapamil was supplied by Eisai Co., Ltd., Tokyo, Japan.

Animals and Tumor Cells. Female BALB/c × DBA/2 (CD2F1) mice weighing 20-23 g were purchased from Charles River Japan, Inc., Tokyo, Japan. P388 and VCR-resistant P388 (P388/VCR) cell lines were supplied by the National Cancer Institute, NIH, Bethesda, MD. The human myelogenous leukemia cell line (K562) was provided by Dr. Ezaki, Cancer Chemotherapy Center, Tokyo, Japan, and its ADM-resistant (K562/ADM) subline was established in our laboratory (7). The human ovarian carcinoma cell line A2780 and its ADM-resistant subline (2780AD) were provided by Drs. R. Ozols and T. Hamilton, Medicine Branch, National Cancer Institute, NIH (17). A cloned human epidermal carcinoma cell line (KB3-1) and its colchicine-resistant subline (KBC-4) were provided by Dr. I. Pastan, National Cancer Institute, NIH (23).

Cell Culture and Drug Treatment. P388 and P388/VCR ascites cells were harvested from a tumor-bearing CD2F1 mouse and maintained in plastic dishes (Corning Glass Works, Corning, NY) in RPMI-1640 supplemented with 5% fetal bovine serum (Grand Island, NY), 20 μM 2-mercaptoethanol, and kanamycin (100 μg/ml). K562, K562/ADM, A2780, 2780AD, KB3-1, and KBC-4 cells were maintained in plastic dishes in RPMI-1640 supplemented with 5% fetal bovine serum and kanamycin (100 μg/ml). For the in vitro drug treatment experiments, tumor cells (1-2 x 10⁷) were seeded in 0.1 ml of culture medium/well in 96-well plates (Corning Glass Works). The cells were treated in triplicate with graded concentrations of antitumor agents in the absence or presence of either MS-073 or verapamil and incubated in a CO₂ incubator at 37°C for 72 h. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cytotoxicity assay was used to measure the cytotoxic effect (24).

Evaluation of Antitumor Activity. One million P388/VCR cells were transplanted i.p. into CD2F1 mice. VCR and MS-073 or verapamil were dissolved in 0.9% NaCl solution (if necessary, MS-073 or verapamil was suspended in 0.9% NaCl solution containing 0.1% carboxymethyl cellulose). VCR and MS-073 or verapamil were mixed and the mixture was administered i.p. daily for 5 days starting from the day after the tumor inoculation (Day 1). In another experiment, MS-073 was administered p.o. or i.v. twice a day and VCR was given i.p. daily for 5 days starting from Day 1. The first administration of MS-073 or verapamil was given just before i.p. injection of VCR, and the second one was given approximately 6 h later. Five or six mice were used for each experimental group. Antitumor activity was evaluated by the mean survival time of the experimental groups and also expressed by the T/C value.

Cellular Accumulation of [3H]VCR. Cell suspensions of K562 and K562/ADM (1.5 x 10⁶/ml) in the growth medium with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer were incubated at 37°C for 2 h. Viability was measured by the Trypan blue exclusion method. Photolabeling of P-glycoprotein with 200 nM [3H]azidopine in K562/ADM plasma membranes was completely inhibited by 10 μM MS-073, indicating that MS-073 reverses MDR by competitively inhibiting drug binding to P-glycoprotein.

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1 To whom requests for reprints should be addressed.
2 The abbreviations used are: MDR, multidrug resistance; VCR, vincristine; ADM, Adriamycin; VP-16, etoposide; ACD, actinomycin D; T/C value, mean survival time of treated mice divided by mean survival time of control mice; IC₅₀, concentration producing 50% inhibition.

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37°C with 30 nM [3H]VCR (5.6 Ci/mmol) in the presence (3 μM) or absence of either MS-073 or verapamil. At various intervals, the amount of intracellular [3H]VCR was determined as described previously (25). In brief, after the resuspension, 0.5-ml aliquots were transferred onto a microtiter plate consisting of Toray Silicon SH550 (Toray Silicon Co., Ltd., Tokyo, Japan) and liquid paraffin (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) at a volume ratio of 4:1 in a 1.5-ml microtiter. After centrifugation, the supernatant fluid was removed. The cell pellet was then lyed overnight with 0.25 ml of 0.5 N KOH and the radioactivity was quantified in a Beekman LS1701 liquid scintillation system after the addition of 10 ml of Aquasol (New England Nuclear, Boston, MA).

Preparation and Photoaffinity Labeling of Plasma Membranes. Preparations of the plasma membrane from K562 or K562/ADM cells and subsequent photoaffinity labeling with [3H]azidopine in the presence or absence of either MS-073 or verapamil were performed as described previously (26). In brief, cells were washed and disrupted with a Dounce homogenizer. The homogenate was then centrifuged at 1000 x g for 10 min. The supernatant was overlaid on 35% sucrose solution and centrifuged for 60 min at 18,000 x g. The membrane fraction at the interface was then centrifuged for 60 min at 100,000 x g. Pellets were resuspended and stored at −70°C until use. The plasma membranes (50 μg of protein) were photolabeled in 40 mM Tris-HCl buffer (pH 7.2), containing 4% dimethyl sulfoxide and 200 nM [3H]azidopine in a final volume of 50 μl in the absence or presence of either MS-073 or verapamil. Photolabeled membranes were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis using gradient gels (4–20%). A total of 12 μg of protein was loaded onto each lane. The gel was fixed, treated with the fluorographic reagent Amplify (Amersham Japan, Ltd.), dried, and then exposed for 10 days at −70°C using Kodak XAR-5 film.

RESULTS

Circumvention of VCR Resistance in P388/VCR Cells by MS-073. The sensitivities of P388 and P388/VCR cells to VCR and the potentiating effects of MS-073 and verapamil on VCR cytotoxicity are illustrated in Fig. 2. The IC50 values of VCR for P388 and P388/VCR cells were 0.75 and 22.0 ng/ml, respectively, indicating an approximate 30-fold resistance. When MS-073 was added at a final concentration of 0.1 μM to P388/VCR cell cultures, the IC50 of VCR shifted from 22.0 to 0.46 ng/ml (Fig. 2A). This result indicates that MS-073 completely overcame VCR resistance of P388/VCR cells at 0.1 μM in vitro. On the other hand, verapamil at 0.1 μM minimally enhanced the cytotoxicity of VCR for P388/VCR cells (Fig. 2B). Under identical experimental conditions MS-073 or verapamil alone had no cytotoxicity for P388/VCR cells up to 1.0 μM. The IC50 value of P388/VCR cells to MS-073 was 4.8 μg/ml (data not shown).

Circuitvention of Drugs Resistance in Various Multidrug-resistant Human Cell Lines. The effects of MS-073 and verapamil on the sensitivities of K562/ADM, 2780AD, and KBC-4 cells to antitumor agents were assayed in the same manner as described previously (27). MS-073 or verapamil alone had no cytotoxicity for these multidrug-resistant cells. Verapamil at 3 mM also completely overcame VCR resistance of P388/VCR cells at 0.1 mM (Fig. 2B). Under identical experimental conditions MS-073 or verapamil alone had no cytotoxicity for P388/VCR cells up to 1.0 μM. The IC50 value of P388/VCR cells to MS-073 was 4.8 μg/ml (data not shown).

Effect of MS-073 upon Potentiating Activity of VCR in P388/VCR-bearing Mice. VCR administered i.p. daily for 5 days starting from Day 1 slightly increased the life span of P388/VCR-bearing mice. The T/C value was 109% at a VCR dosage of 100 μg/kg. Coadministration of MS-073 with VCR increased the life span of the P388/VCR bearer (Fig. 3). T/C values of 109–150% were obtained when MS-073 (3–100 mg/kg) was administered with VCR (100 μg/kg). MS-073 alone did not significantly prolong the life span of P388/VCR-bearing mice. VCR administered i.p. daily for 5 days starting from Day 1, and MS-073 coadministered with VCR in the absence or presence of either MS-073 or verapamil were performed as described previously (26).

Preparation and Photoaffinity Labeling of Plasma Membranes. Preparations of the plasma membrane from K562 or K562/ADM cells and subsequent photoaffinity labeling with [3H]azidopine in the presence or absence of either MS-073 or verapamil were performed as described previously (26).

Table 1. Effect of MS-073 on drug resistance in MDR cells

Tumor cells (1–2 x 10^6) were seeded in 0.1 ml of culture medium and then treated with graded concentrations of antitumor agents in the absence or presence (3 μM) of either MS-073 (A) or verapamil (B). Drug concentrations of MS-073 and verapamil were 0 ( ), 0.1 ( ), 0.3 ( ), and 1.0 μM ( ).

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Modifier</th>
<th>Relative resistance to antitumor agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>K562</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>K562/ADM</td>
<td>None</td>
<td>381</td>
</tr>
<tr>
<td></td>
<td>MS-073 (3 μM)</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Verapamil (3 μM)</td>
<td>10</td>
</tr>
<tr>
<td>A2780</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>2780AD</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MS-073 (3 μM)</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Verapamil (3 μM)</td>
<td>6</td>
</tr>
<tr>
<td>KB3-1</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>KBC-4</td>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MS-073 (3 μM)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Verapamil (3 μM)</td>
<td>3</td>
</tr>
</tbody>
</table>

*IC50 of MDR cells with or without modifier was divided by IC50 of their parent cells.
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Fig. 3. Effect of MS-073 compared with verapamil administered i.p. on antitumor activity of VCR in P388/VCR-bearing mice. Each group of five CD2F1 mice was given i.p. implants of 10⁶ cells of P388/VCR leukemia on Day 0. VCR (100 µg/kg) was given i.p. daily from Days 1 to 5 with MS-073 or verapamil at the indicated doses. Point, mean; bar, ±SD.

Fig. 4. Effects of MS-073 compared with verapamil administered i.p. on antitumor activity of VCR at the indicated doses in P388/VCR-bearing mice. Each group of five CD2F1 mice was given i.p. implants of 10⁶ cells of P388/VCR leukemia on Day 0. VCR at the indicated doses was given i.p. daily from Days 1 to 5 with MS-073 (30 mg/kg) or verapamil (100 mg/kg). Point, mean; bar, ±SD.

Fig. 5. Effect of MS-073 administered p.o. on antitumor activity of VCR in P388/VCR-bearing mice. Each group of six CD2F1 mice was given i.p. implants of 10⁶ cells of P388/VCR leukemia on Day 0. MS-073 was administered p.o. twice/day and VCR (100 µg/kg) was given i.p. daily at the time of the first injection of MS-073 each day from Days 1 to 5. Point, mean; bar, ±SD.

show any therapeutic effect at 100 mg/kg. An increase in the T/C value was also observed in P388/VCR-bearing mice when VCR was given with verapamil (Fig. 3). The dose of verapamil could be increased to 100 mg/kg without manifestation of toxicity in combination with VCR, while 125 mg/kg verapamil was toxic (data not shown). At a verapamil dose of 100 mg/kg, a T/C value of 127% was obtained with VCR (100 µg/kg). MS-073 at 30 and 100 mg/kg showed a life-prolongation effect in combination with VCR which could not be obtained at any dose of verapamil tested. T/C values of 141 and 150% were obtained at 30 and 100 mg/kg of MS-073 with VCR (100 µg/kg), respectively. These results indicate that MS-073 shows significant effect at nontoxic doses, as compared to verapamil, in this type of combination treatment. In the repeated experiments, T/C values of 140–150% were obtained at an MS-073 dose of 100 mg/kg with VCR (100 µg/kg).

In another experiment, graded doses of VCR were used in combination with MS-073 (30 mg/kg) (Fig. 4). T/C values of 115–143% were obtained with VCR (3–100 µg/kg), although VCR at 300 µg/kg was slightly toxic in this combination treatment. Life-prolongation effects were also observed when verapamil (100 mg/kg) was combined with VCR (10 or 30 µg/kg). However, these effects were less than those obtained in the combination treatment of MS-073 (30 mg/kg) and VCR (10 or 30 µg/kg). VCR (100 µg/kg) with verapamil (100 mg/kg) was toxic in this experiment.

When MS-073 was administered p.o. twice/day and VCR was given i.p. daily at the time of the first injection of MS-073 each day for 5 days starting from Day 1, the life span of P388/VCR-bearing mice was increased. At MS-073 doses of 400–750 mg/kg given twice (total dose, 800–1500 mg/kg/day), T/C values of 159–166% were obtained with VCR (100 µg/kg) (Fig. 5). MS-073 at 1000 mg/kg given twice (total dose, 2000 mg/kg/day) with VCR (100 µg/kg) was slightly toxic. VCR alone at 100 µg/kg showed a T/C value of 104% in this experiment. Verapamil administered p.o. slightly enhanced the antitumor activity of VCR in P388/VCR-bearing mice (data not shown). A T/C value of 111% was obtained at a verapamil dose of 100 mg/kg given twice (total dose, 200 mg/kg/day) with VCR (100 µg/kg). Verapamil at 200 mg/kg given twice (total dose, 400 mg/kg/day), however, was toxic.

MS-073 administered i.v. also significantly enhanced the antitumor activity of VCR. At MS-073 doses of 15 mg/kg given twice (total dose, 30 mg/kg/day) and 30 mg/kg given twice (total dose, 60 mg/kg/day), T/C values of 125 and 131% were obtained with VCR (100 µg/kg), respectively (data not shown). MS-073 at 45 mg/kg given twice (total dose, 90 mg/kg/day) with VCR (100 µg/kg) was slightly toxic. MS-073 at 60 mg/kg given twice (total dose, 120 mg/kg/day) with VCR (100 µg/kg) was slightly toxic.

Effect of MS-073 upon Antitumor Activity of VCR in P388-bearing Mice. The combined effects of VCR and MS-073 were also investigated against the parent P388-bearing mice (Table 2). T/C values of 121–166% were obtained when VCR (10–100 µg/kg) alone was administered i.p. daily from Days 1 to 5. Coadministration of MS-073 with VCR increased the life span of the P388-bearing mice. Increases in life span of 30–100%
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Table 2 Effect of MS-073 on antitumor activity of VCR in P388-bearing mice

<table>
<thead>
<tr>
<th>Drug and dosage</th>
<th>Survival time (days)</th>
<th>T/C (%)</th>
<th>T/V/(^{a}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.0 ± 0.7</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>MS-073 (100 mg/kg)</td>
<td>9.0 ± 1.7</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>VCR (100 µg/kg)</td>
<td>14.9 ± 1.0</td>
<td>166</td>
<td>100</td>
</tr>
<tr>
<td>+MS-073 (100 mg/kg)</td>
<td>17.9 ± 0.7</td>
<td>199</td>
<td>120</td>
</tr>
<tr>
<td>+VCR (100 µg/kg)</td>
<td>16.6 ± 1.7</td>
<td>184</td>
<td>111</td>
</tr>
<tr>
<td>+VCR (30 µg/kg)</td>
<td>12.6 ± 0.6</td>
<td>140</td>
<td>100</td>
</tr>
<tr>
<td>+MS-073 (100 mg/kg)</td>
<td>16.7 ± 3.9</td>
<td>186</td>
<td>133</td>
</tr>
<tr>
<td>+VCR (30 µg/kg)</td>
<td>13.9 ± 0.5</td>
<td>154</td>
<td>110</td>
</tr>
<tr>
<td>+MS-073 (30 mg/kg)</td>
<td>10.9 ± 0.8</td>
<td>121</td>
<td>100</td>
</tr>
<tr>
<td>+VCR (100 µg/kg)</td>
<td>12.2 ± 0.6</td>
<td>136</td>
<td>112</td>
</tr>
<tr>
<td>+MS-073 (30 mg/kg)</td>
<td>11.3 ± 0.6</td>
<td>126</td>
<td>104</td>
</tr>
</tbody>
</table>

* T/V at each VCR dosage, the mean survival time of the treated group divided by the mean survival time of the group of mice treated with VCR alone.

\(^{a}\) Mean ± SD.

were obtained at MS-073 doses of 30 or 100 mg/kg with VCR (10-100 µg/kg). These values were 10-30% higher as compared to the effects of VCR alone (see T/V value in the Table 2).

Effect of MS-073 on \[^{3}H\]VCR Accumulation in K562/ADM Cells. Cellular accumulation of VCR was examined in the presence of 30 nm \[^{3}H\]VCR (Fig. 6). Accumulation of \[^{3}H\]VCR in cultured K562 cells increased with time under conditions of constant drug exposure. By 4 h, 6.3 pmol of VCR was found in 10<sup>6</sup> K562 cells. VCR was not found to accumulate in K562/ADM cells. MS-073 added to the culture at 3 µM greatly increased the amount of cellular VCR in K562/ADM cells. The amount of VCR in 10<sup>6</sup> K562/ADM cells treated with MS-073 (3 µM) was 3.6 pmol at 4 h, while 1.4 pmol of VCR was found at the same incubation time in 10<sup>6</sup> K562/ADM cells treated with verapamil (3 µM).

Inhibition of \[^{3}H\]Azidopine Photolabeling of P-glycoprotein by MS-073. Azidopine, a phototactic analogue of dihydropyridine, photolabels P-glycoprotein in plasma membranes of multidrug-resistant cells and the labeling is inhibited by vinblastine and some calcium channel blockers (27, 28). We also reported that a phototactic analogue of verapamil photolabels P-glycoprotein in the plasma membrane prepared from K562/ADM cells (26). By using this photolabeling system, we investigated whether MS-073 inhibited the \[^{3}H\]azidopine photolabeling of P-glycoprotein. As shown in Fig. 7, \[^{3}H\]azidopine specifically labeled an M, 170,000-180,000 protein of K562/ADM but not in drug-sensitive K562 cells. In the presence of 10 µM MS-073, the radiolabeling of P-glycoprotein was almost completely inhibited (95% inhibition), while in the presence of 10 or 100 µM verapamil the radiolabeling was partially inhibited (18 or 50% inhibition, respectively). This result shows that the inhibitory effect of MS-073 is much stronger than that of verapamil.

DISCUSSION

MS-073 could completely overcome resistance of P388/VCR cells to VCR and also remarkably enhanced the cytotoxicity of VCR, ADM, VP-16, and ACD in multidrug-resistant human cell lines in vitro. Previously, we reported that calcium channel blockers and calmodulin inhibitors could overcome MDR (13-14). Since then various compounds have been reported to overcome drug resistance and some of these compounds could circumvent MDR in vivo (13, 15, 29-33). Although the effects of MS-073 were not compared with those of other chemosensitizers except for verapamil, we usually needed relatively high doses of the chemosensitizers to obtain the effects in vivo. In this report, we showed that MS-073 administered i.p. in combination with VCR increased the T/C ratio in a dose-dependent manner. The effect of MS-073 at a dose of 3 mg/kg was almost equal to that obtained by verapamil at a dose of 25 mg/kg, and MS-073 at 10-100 mg/kg showed a life-prolongation effect which could not be obtained at any dose of verapamil tested. These results indicate that MS-073 could show superior effect at relatively low doses in vivo as well as in vitro, as compared to other chemosensitizers. MS-073 administered p.o. also enhanced T/C ratios when combined with chemotherapy. A T/C value of approximately 160% was obtained at MS-073 doses of

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discussions. We also thank A. Yamazaki for her technical assistance and arrhythmias. MS-073 had a much less hypotensive action of chemotherapy with calcium channel blockers and structurally related compounds induces side effects such as hypotension and arrhythmias. MS-073 had a much less hypotensive action in dogs than verapamil and the 50% lethal dose of MS-073 against mice was more than 1000 mg/kg when MS-073 was injected i.p. (data not shown). These results indicate that MS-073 is a potential candidate to be applied clinically in this type of combination chemotherapy.

Recently, we reported that verapamil competitively inhibits VCR binding to the high affinity-binding sites of VCR on K562/ADM plasma membrane (34) and a phototoxic analogue of verapamil photolabels P-glycoprotein in the plasma membrane prepared from K562/ADM cells (26). These results suggest that verapamil enhances the retention of antitumor agents through competition for closely related binding sites on P-glycoprotein. In order to clarify by what mechanism MS-073 reverses MDR, we examined the effect of MS-073 on [3H]VCR accumulation in K562/ADM cells and [3H]azidopine photolabeling of P-glycoprotein in the plasma membrane prepared from K562/ADM cells. MS-073 strongly enhanced VCR accumulation in K562/ADM cells and completely inhibited the [3H]azidopine photolabeling of P-glycoprotein at 10 μM. These results indicate that the mechanism by which MS-073 reverses MDR is similar to that of verapamil. The effect of MS-073 on the enhancement of VCR accumulation and on the inhibition of azidopine photolabeling was remarkably stronger than that of verapamil. This fact might explain the superior effect of MS-073 on the reversal of MDR both in vitro and in vivo as compared to verapamil. Further examination of MS-073 is warranted.

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