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Reversal of multidrug resistance by a novel quinoline derivative, MS-209

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Abstract MS-209, a novel quinoline derivative, was examined for its reversing effect on multidrug-resistant tumor cells. MS-209 at 1–10 μ*M* completely reversed resistance against vincristine (VCR) in vitro in multidrug-resistant variants of mouse leukemia P388 cells (VCR-resistant P388/VCR and Adriamycin (ADM)-resistant P388/ADM) and human leukemia K562 cells (VCR-resistant K562/VCR and ADM-resistant K562/ADM). MS-209 at 1–10 μ*M* also completely reversed resistance against ADM in vitro in P388/VCR cells, K562/VCR cells, and K562/ADM cells. In ADM-resistant P388 (P388/ADM) cells, however, ADM resistance was only partially reversed at the MS-209 concentrations tested. MS-209 enhanced the chemotherapeutic effect of VCR in P388/VCR-bearing mice. When MS-209 was given p.o. at 80 mg/kg twice a day (total dose, 160 mg/kg per day) with 100 μg/kg VCR, a treated/control (T/C) value of 155% was obtained. MS-209 also enhanced the chemotherapeutic effect of ADM in P388/ADM-bearing mice. The most prominent effects were obtained when MS-209 was given with 2 mg/kg ADM, yielding T/C values of 150%–194% for the combined treatment at an MS-209 dose of 200–450 mg/kg. MS-209 inhibited [^{3}H]-azidopine photolabeling of P-glycoprotein efficiently. Furthermore, the accumulation of ADM in K562/ADM cells was increased more efficiently by MS-209 than by verapamil. These results indicate that MS-209, like verapamil, directly interacts with P-glycoprotein and inhibits the active efflux of antitumor agents, thus overcoming multidrug resistance in vitro and in vivo.

Key words MS-209 · Quinoline · Multidrug resistance

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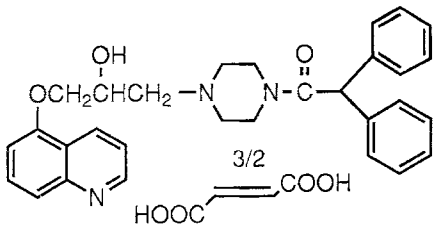
Introduction

Development of specific drug-resistant tumor cells during treatment is a major problem with current cancer chemotherapy. 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 [8, 13, 18]. A major mechanism for this multidrug resistance (MDR) is attributed to the reduced accumulation of antitumor agents in resistant cells [3, 5, 9, 26]. It has been shown that a particular class of transmembrane glycoproteins, called P-glycoprotein, functions as an energy-dependent drug-efflux pump [2, 7, 11, 14, 17].

Since the discovery of a calcium channel blocker, verapamil, as an agent for overcoming MDR, various compounds, including quinidine, tamoxifen, cyclosporine, and others, have been reported to overcome drug resistance [12, 19, 22–25, 27] (see [27] for a review). We have previously reported that a new quinoline derivative, MS-073, is more effective than verapamil in interacting with P-glycoprotein and in reversing MDR [16]. High doses of MS-073, however, were necessary to obtain the effect in combination chemotherapy with vincristine (VCR) when MS-073



$C_{30}H_{31}N_3O_3 \cdot 3/2C_4H_4O_4$

Fig. 1 Chemical structure of MS-209

was given orally. In our efforts to find more effective drugs from newly synthesized quinoline compounds with fewer side effects, another quinoline compound, MS-209, was selected as the most effective MDR-reversing agent for oral administration in combination chemotherapy with VCR or Adriamycin (ADM) using MDR tumor cells. MS-209 has now entered clinical trials in Japan. In this study, we describe the potency of MS-209 in overcoming MDR in experimental models.

Materials and methods

Drugs

VCR was purchased from Shionogi Co., Ltd. (Osaka, Japan); ADM, from Kyowa Hakko Co., Ltd. (Tokyo, Japan); [^{14}C]-ADM (57 mCi/mmol) and [^{3}H]-azidopine (50 Ci/mmol), from Amersham Japan Ltd. (Tokyo, Japan); and verapamil, from Eisai Co., Ltd. (Tokyo, Japan). MS-209 (Fig. 1) was synthesized in the Life Science Laboratory, Mitsui Toatsu Chemicals Inc. (Chiba, Japan) [6].

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, VCR-resistant P388 (P388/VCR), and ADM-resistant P388 (P388/ADM) cell lines were supplied by the National Cancer Institute (NIH, Bethesda, Md.). The K562 cell line was provided by Dr. Ezaki, Cancer Chemotherapy Center (Tokyo, Japan), and its VCR-resistant (K562/VCR) and ADM-resistant (K562/ADM) sublines were established in the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research (Tokyo, Japan).

Cell culture and drug treatment

P388, P388/VCR, and P388/ADM ascites cells were harvested from tumor-bearing CD2F1 mice and maintained in plastic dishes (Corning Glass Works, Corning, N.Y.) in RPMI-1640 medium supplemented with 10% fetal bovine serum, 20 μ*M* 2-mercaptoethanol, and kanamycin (100 μg/ml). K562, K562/VCR, and K562/ADM cells were maintained in plastic dishes in RPMI-1640 medium supplemented with 10% fetal bovine serum and kanamycin (100 μg/ml). For the in vitro drug treatment experiments, tumor cells (0.5–1 × 10^3) 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 MS-209 and were then incubated in a CO_2 incubator at 37°C for 72 h. The 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay was used to measure the cytotoxic effect [1]. The median concentration of drug necessary to inhibit the growth of tumor cells by 50% (IC_{50}) was determined by plotting the logarithm of the drug concentration versus the growth rate (percentage of control) of the treated cells.

Evaluation of antitumor activity

P388/VCR and P388/ADM cells were transplanted i.p. into CD2F1 mice (10^6 cells/mouse). VCR and ADM were dissolved in 0.9% NaCl solution and MS-209 was suspended in 0.9% NaCl solution containing 0.1% Tween 80 (Wako Pure Chemical Industries, Ltd., Tokyo, Japan). MS-209 was given p.o. once or twice a day, and VCR or ADM was given i.p. daily for 5 days starting on the day following tumor inoculation. The first administration of the test compound was given before i.p. injection of VCR, and the second was given approximately 6 h later. In combination chemotherapy with ADM, the test compound was given only once a day before i.p. injection of ADM. Six mice were used for each experimental group. Antitumor activity was evaluated by the median survival time of the experimental group and was expressed as the treated/control (T/C) value.

Cellular accumulation of [^{14}C]-ADM

Suspensions of K562 and K562/ADM cells (1 × 10^6/ml) in growth medium containing 10 m*M* 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid buffer were incubated at 37° or 0°C with 50 n*M* [^{14}C]-ADM (57 mCi/mmol) in the presence (3 μ*M*) or absence of either MS-209 or verapamil. At various intervals, the amount of intracellular [^{14}C]-ADM was determined as described previously [20]. In brief, 0.5-ml aliquots were transferred onto an oil layer consisting of Toray Silicon SH550 and oil paraffin (4:1) in a 1.5-ml microtube. After centrifugation, the supernatant fluid was removed. The cells comprising the pellet were then lysed with 0.25 ml of 0.5 *N* KOH and the radioactivity was counted by a liquid scintillation system.

Preparation and photoaffinity labeling of plasma membranes

Preparation of plasma membranes from K562 or K562/ADM cells and subsequent photolabeling with [^{3}H]-azidopine in the presence or absence of MS-209 was performed as described previously [29]. In brief, cells were washed and disrupted with a Dounce homogenizer. The homogenate was then centrifuged at 1,000 *g* for 10 min. The supernatant was overlaid on 35% sucrose and centrifuged for 60 min at 18,000 *g*. The membrane fraction at the interface was then centrifuged for 60 min at 100,000 *g*. Pellets were resuspended and stored at − 70°C until use. The plasma membranes (50 μg of protein) were photolabeled in 40 m*M* Tris-HCl buffer (pH 7.2) containing 4% dimethylsulfoxide and 200 n*M* [^{3}H]-azidopine in a final volume of 50 μl in the presence or absence of MS-209. 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 to Kodak XAR-5 films at − 70°C for 10 days.

Table 1 Enhancement of VCR cytotoxicity by MS-209 in MDR cells[a]

MS-209	IC_{50}(ng/ml) of VCR[b]					
(μ*M*)	P388	P388/VCR	P388/ADM	K562	K562/VCR	K562/ADM
0.0	1.89 (1)	54.5 (1)	75.5 (1)	0.34 (1)	58.2 (1)	628 (1)
0.1	0.64 (3.0)	19.2 (2.8)	53.9 (1.4)	0.34 (1)	19.5 (3.0)	608 (1.0)
0.3	0.60 (3.2)	6.26 (8.7)	24.3 (3.1)	0.24 (1.4)	4.04 (14)	433 (1.5)
1.0	0.41 (4.6)	1.48 (81)	4.72 (16)	0.20 (1.7)	0.88 (66)	19.3 (33)
3.0	0.18 (11)	0.20 (273)	0.92 (82)	0.17 (2.0)	0.55 (106)	2.27 (277)
10.0	< 0.1 (> 19)	–	–	–	0.21 (277)	0.36 (1744)

[a] Tumor cells were seeded in 0.1 ml of culture medium and then treated with graded concentrations of antitumor agents in the absence or presence of MS-209. After 72 h of continuous drug exposure, growth-inhibitory effects were evaluated by MTT assay [1]
[b] Each value represents the mean of triplicate determinations. Standard deviations were within 10% of each value. Numbers in parentheses represent relative values as compared with the IC_{50} obtained for each parent cell line in the absence of MS-209

Table 2 Enhancement of ADM cytotoxicity by MS-209 in MDR cells[a]

MS-209	IC_{50} (ng/ml) of ADM[b]					
(μ*M*)	P388	P388/VCR	P388/ADM	K562	K562/VCR	K562/ADM
0.0	5.70 (1)	35.1 (1)	385 (1)	6.53 (1)	62.4 (1)	1,405 (1)
0.1	5.39 (1.1)	17.3 (2.0)	252 (1.5)	6.17 (1.0)	36.6 (1.7)	1,323 (1.1)
0.3	4.85 (1.2)	10.2 (3.4)	168 (2.3)	5.86 (1.1)	18.7 (3.3)	649 (2.2)
1.0	3.03 (1.9)	4.27 (8.2)	50.8 (7.6)	5.80 (1.1)	8.57 (7.3)	42.5 (33)
3.0	2.54 (2.2)	1.76 (20)	20.8 (19)	4.94 (1.3)	6.14 (10)	13.4 (105)
10.0	1.02 (5.6)	–	–	–	4.88 (13)	6.29 (223)

[a] Tumor cells were seeded in 0.1 ml of culture medium and then treated with graded concentrations of antitumor agents in the absence or presence of MS-209. After 72 h of continuous drug exposure, growth-inhibitory effects were evaluated by MTT assay [1]
[b] Each value represents the mean of triplicate determinations. Standard deviations were within 10% of each value. Numbers in parentheses represent relative values as compared with the IC_{50} obtained for each parent cell line in the absence of MS-209

Results

Circumvention of VCR and ADM resistance in MDR cell lines

We examined the effect of MS-209 on the sensitivity of various MDR cells and their parental cells to VCR. As shown in Table 1, P388/VCR and P388/ADM cells showed 29- and 40-fold greater resistance, respectively, to VCR as compared with parental P388 cells. When MS-209 was added at a final concentration of 0.1–10 μ*M* to the MDR P388 cells, MS-209 at 1 and 3 μ*M* completely reversed VCR resistance in P388/VCR and P388/ADM cells, respectively. The sensitivity of the parental P388 cells to VCR was moderately enhanced by MS-209; 11-fold sensitization was observed at 3 μ*M* MS-209. MS-209 alone at 10 μ*M* had a cytotoxic effect on P388/VCR and P388/ADM cells. MS-209 also markedly enhanced the sensitivity to VCR of the MDR K562 cells. K562/VCR and K562/ADM cells showed 171- and 1,847-fold resistance, respectively, to VCR as compared with parental K562 cells. VCR resistance in MDR K562 cells was completely reversed by 10 μ*M* MS-209. MS-209 at 10 μ*M* had a cytotoxic effect on K562 cells but not on MDR K562 cells.

We also examined the potentiating effect of MS-209 on ADM in the MDR P388 and K562 cells (Table 2). P388/VCR, P388/ADM, K562/VCR, and K562/ADM cells showed 6-, 68-, 10-, and 215-fold resistance, respectively, to ADM as compared with their respective parental cells. ADM resistance in P388/VCR, K562/VCR, and K562/ADM cells was completely reversed by MS-209 at 1, 3, and 10 μ*M*, respectively. In P388/ADM cells, 19-fold sensitization was observed at 3 μ*M* MS-209 but complete reversal was not attained. MS-209 alone at 10 μ*M* had a cytotoxic effect on P388/VCR, P388/ADM, and K562 cells. The sensitivity of the parental P388 cells to ADM was moderately enhanced by MS-209.

Combined chemotherapeutic effect of VCR and MS-209 on P388/VCR-bearing mice

VCR (100 μg/kg) alone given i.p. daily for 5 days starting on day 1 had no significant chemotherapeutic effect on P388/VCR-bearing mice (Table 3). When MS-209 was given p.o. twice a day and VCR was given

Table 3 Effect of MS-209 on the antitumor activity of VCR in P388/VCR-bearing mice[a]

Treatment	*n*	Median (days)	Range (days)	T/C[b] (%)	Body weight change[c] (g)
Control	12	11.0	9–13	100	− 0.2
MS-209 (110 mg/kg × 2)	6	11.5	9–14	106	− 0.1
VCR (100 μg/kg)	6	11.5	10–13	105	− 1.4
+ MS-209 (25 mg/kg × 2)	6	12.0	11–13	109	− 1.2
+ MS-209 (50 mg/kg × 2)	6	13.0	9–15	118	− 2.5
+ MS-209 (80 mg/kg × 2)	6	17.0	15–18	155	− 2.8
+ MS-209 (110 mg/kg × 2)	6	7.5	7–8	68	− 2.6

[a] CD2F1 mice were given i.p. implants of 10^6 P388/VCR leukemia cells on day 0. MS-209 was given p.o. twice a day and VCR (100 μg/kg) was given i.p. daily with the first administration of MS-209 from day 1 to day 5
[b] T/C value: median survival time of treated mice divided by that of control mice
[c] Difference in body weight (g) between days 5 and 1

Table 4 Effect of MS-209 on the antitumor activity of ADM in P388/ADM-bearing mice[a]

Treatment	*n*	Median (days)	Range (days)	T/C[b] (%)	Body weight change[c] (g)
Experiment 1:					
Control	12	9.0	8–12	100	+ 1.0
ADM (1 mg/kg)	6	9.0	8–11	100	+ 0.4
+ MS-209 (50 mg/kg)	6	10.0	9–12	111	+ 0.5
+ MS-209 (100 mg/kg)	6	10.0	9–11	111	+ 0.5
+ MS-209 (200 mg/kg)	6	11.0	10–15	122	+ 0.6
ADM (2 mg/kg)	6	9.5	8–12	106	+ 0.2
+ MS-209 (50 mg/kg)	6	10.0	10–11	111	+ 0.1
+ MS-209 (100 mg/kg)	6	11.0	10–12	122	− 0.2
+ MS-209 (200 mg/kg)	6	13.0	11–15	144	− 0.1
ADM (3 mg/kg)	6	10.0	8–11	111	− 0.5
+ MS-209 (50 mg/kg)	6	10.5	10–15	117	− 0.7
+ MS-209 (100 mg/kg)	6	11.0	10–12	122	− 0.9
+ MS-209 (200 mg/kg)	6	11.5	11–13	128	− 1.2
ADM (4 mg/kg)	6	9.5	8–12	106	− 0.8
ADM (6 mg/kg)	6	8.5	8–11	94	− 1.7
ADM (8 mg/kg)	6	8.0	8–8	89	− 2.5
Experiment 2:					
Control	12	9.0	9–11	100	+ 1.0
ADM (2 mg/kg)	6	11.0	10–12	122	− 0.3
+ MS-209 (200 mg/kg)	6	13.5	12–20	150	+ 0.3
+ MS-209 (300 mg/kg)	6	17.5	13–21	194	− 1.1
+ MS-209 (450 mg/kg)	6	15.5	13–17	172	− 1.9

[a] CD2F1 mice were given i.p. implants of 10^6 P388/VCR leukemia cells on day 0. MS-209 was given p.o. once a day just before i.p. injection of ADM from day 1 to day 5
[b] T/C value: median survival time of treated mice divided by that of control mice
[c] Difference in body weight (g) between days 5 and 1

i.p. daily for 5 days starting on day 1 at the time of the first injection of MS-209, the life span of P388/VCR-bearing mice was significantly increased. The most prominent result was observed at an MS-209 dose of 80 mg/kg given twice a day (total dose, 160 mg/kg per day) with 100 μg/kg VCR, whereby the T/C value was 155%. MS-209 alone given at 110 mg/kg twice a day (total dose, 220 mg/kg per day) showed no significant chemotherapeutic effect. The same dose of MS-209 given with 100 μg/kg VCR was presumably toxic to the mice and resulted in a decreased life span in comparison with that of control animals.

Combined chemotherapeutic effect of ADM and MS-209 on P388/ADM-bearing mice

As shown in Table 4, ADM (1–8 mg/kg) alone given i.p. daily for 5 days starting on day 1 had no significant chemotherapeutic effect on P388/ADM-bearing mice in experiment 1, although ADM at 2 mg/kg slightly increased the life span of mice in experiment 2, giving a T/C value of 122%. These in vivo results confirm the high resistance of P388/ADM cells to ADM observed in in vitro experiments. Oral administration of MS-209 with ADM given i.p. once a day for 5 consecutive days

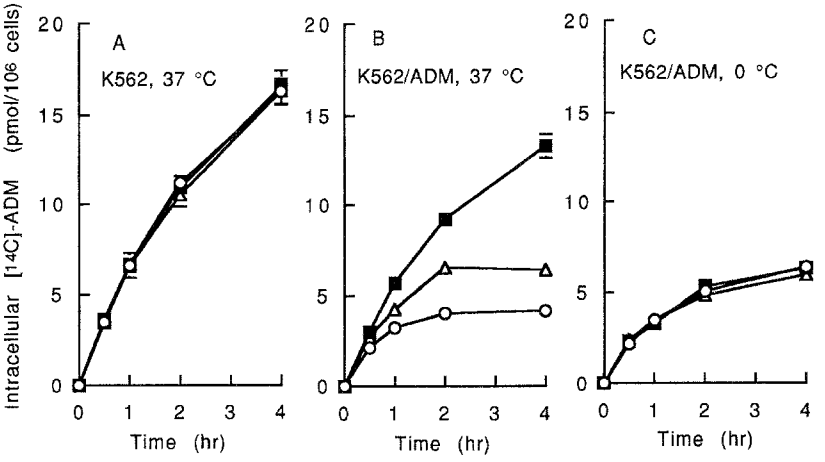


Fig. 2A–C Effect of MS-209 on the uptake of [^{14}C]-ADM in K562 and K562/ADM leukemia cells. Suspensions of K562 and K562/ADM cells (1×10^6/ml) in growth medium containing 10 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer were incubated at 37° or 0°C with 50 n*M* [^{14}C]-ADM (57 mCi/mmol) in the absence (○) or presence (3 μ*M*) of either MS-209 (■) or verapamil (△). At various intervals, the amount of intracellular [^{14}C]-ADM was determined as described previously [20]

starting on day 1 apparently increased the life span of P388/ADM-bearing mice. The most prominent results were obtained when MS-209 was given with 2 mg/kg ADM. T/C values of 111%–144% and 150%–194% were obtained with the combined treatment at MS-209 doses of 50–200 (experiment 1) and 200–450 (experiment 2) mg/kg, respectively.

Effect of MS-209 on the cellular accumulation of [^{14}C]-ADM

The intracellular accumulation of [^{14}C]-ADM in tumor cells was examined with and without the addition of MS-209 to the culture medium. [^{14}C]-ADM efficiently accumulated in K562 cells at 37°C, and MS-209 and verapamil at 3 μ*M* had no effect on [^{14}C]-ADM accumulation (Fig. 2A). In K562/ADM cells, the accumulation of [^{14}C]-ADM was extremely reduced at 37°C (Fig. 2B). MS-209 at 3 μ*M* enhanced [^{14}C]-ADM accumulation in K562/ADM cells to an extent almost comparable with that observed in parental K562 cells incubated without MS-209 (Fig. 2B). Verapamil at 3 μ*M* caused moderate enhancement of [^{14}C]-ADM accumulation in K562/ADM cells (Fig. 2B). When the cells were incubated at 0°C to suppress the intracellular energy system, no potentiation of [^{14}C]-ADM accumulation by either MS-209 or verapamil was observed (Fig. 2C).

Inhibition of [^{3}H]-azidopine photolabeling of P-glycoprotein by MS-209

Azidopine, a photoactive analog of dihydropyridine, photolabels P-glycoprotein in plasma membranes of MDR cells; this labeling is inhibited by vinblastine and some calcium channel blockers [15, 28]. We have also reported that a photoactive analog of verapamil photolabels P-glycoprotein in the plasma membranes of K562/ADM cells [29]. Using this photolabeling

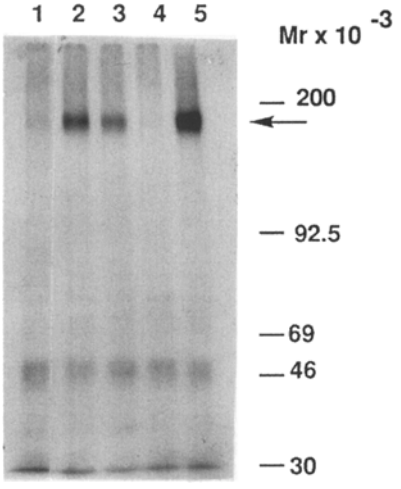


Fig. 3 Inhibition of [^{3}H]-azidopine photolabeling of P-glycoprotein. K562 (*lane* 1) and K562/ADM (*lanes* 2–5) membrane vesicles (50 μg of protein) were incubated with 200 n*M* [^{3}H]-azidopine in the absence (*lanes* 1, 5) or presence of MS-209 at 1 (*lane* 2), 10 (*lane* 3), or 100 μ*M* (*lane* 4). After solubilization, photolabeled proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously [28]

system, we investigated whether MS-209 inhibited the [^{3}H]-azidopine photolabeling of P-glycoprotein. As shown in Fig. 3, [^{3}H]-azidopine specifically labeled a 170,000- to 180,000-Da protein in K562/ADM cells but not in drug-sensitive K562 cells. In the presence of 100 μ*M* MS-209, the radiolabeling of P-glycoprotein was completely inhibited. This result suggests that MS-209 directly interacts with P-glycoprotein and inhibits the transport of antitumor agents.

Discussion

We found that MS-209 completely reversed VCR resistance in vitro in highly resistant K562/ADM cells (1,847-fold resistance) as well as moderately resistant

P388/VCR, P388/ADM, and K562/VCR cells (29-, 40-, and 171-fold resistance, respectively). Human K562/ADM cells were also highly resistant to ADM (215-fold resistance), and complete reversal could be achieved with MS-209. In moderately resistant rodent P388/ADM cells (68-fold resistance), however, ADM resistance was only partially reversed at the MS-209 concentrations tested. This result suggests that rodent P388/ADM cells might have another mechanism of resistance that is unaffected by MS-209. In fact, P388/ADM cells have been reported to show reduced levels of DNA topoisomerase II protein, which could result in resistance to ADM [4].

MS-209 inhibited the [^{3}H]-azidopine photolabeling of P-glycoprotein efficiently. Furthermore, the accumulation of ADM in K562/ADM cells was increased more efficiently by MS-209 than by verapamil. These results suggest that the mechanism of action of MS-209 for reversing MDR is similar to that of verapamil. It has been reported that verapamil binds competitively to the drug-binding site on P-glycoprotein and is transported from resistant cells by a mechanism similar to that of antitumor agents [10, 29].

MS-209 enhanced the cytotoxicity of VCR and ADM in parental P388 cells. Although the amount is small, P388 cells express mdr1b P-glycoprotein. The effect of MS-209 on P388 cells may be due to inhibition of the P-glycoprotein marginally expressed in these cells. Alternatively, MS-209 may have another unknown mechanism to sensitize tumor cells to VCR and ADM.

We have previously reported that MS-073, whose structure is similar to that of MS-209, is far more potent than verapamil in sensitizing MDR cells both in vitro and in vivo [16]. MS-073 given i.p. at lower doses significantly enhanced the chemotherapeutic effect of VCR in P388/VCR-bearing mice. However, when it was given orally, relatively high doses of MS-073 were necessary to obtain the effect in combination chemotherapy with VCR. The most prominent result was obtained at MS-073 doses of 400–750 mg/kg given twice a day (total dose, 800–1,500 mg/kg per day) with 100 μg/kg VCR for 5 consecutive days in P388/VCR-bearing mice. On the other hand, the optimal dose of MS-209 was 80 mg/kg given twice daily (total dose, 160 mg/kg per day), yielding a T/C value of 155%. The T/C value was almost the same as that obtained in combination treatment involving MS-073 given at 800–1,500 mg/kg per day. These results indicate that the oral bioavailability of MS-209 may be superior to that of MS-073. In fact, the blood concentration of MS-209 was almost 10-fold that of MS-073 when the compounds were given orally to mice at the same doses (data not shown).

In P388/ADM-bearing mice, comparatively high doses of MS-209 were necessary to obtain chemotherapeutic effects in combination treatment with ADM relative to the effective dose of MS-209 given in combination with VCR in P388/VCR-bearing mice (Tables 3, 4). These results can be explained in part by the high resistance of P388/ADM cells to ADM. Actually, P388/ADM cells showed 68-fold resistance to ADM in vitro and the ADM resistance was only partially reversed by MS-209 at the concentrations tested (Table 2). On the other hand, 1 μ*M* MS-209 completely reversed the VCR resistance of P388/VCR cells (Table 1). Together with other pharmacological and toxicological profiles (data not shown), the present results suggest that MS-209 will be an orally active MDR-reversing drug.

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