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Circumvention of multidrug resistance by a quinoline derivative, MS-209, in multidrug-resistant human small-cell lung cancer cells and its synergistic interaction with cyclosporin A or verapamil

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Abstract *Purpose and methods*: To develop a clinically useful approach to circumvent P-glycoprotein (P-gp)-mediated multidrug resistance (MDR) in MDR human small-cell lung cancer (SCLC), we examined the ability of a novel quinoline compound, MS-209, to reverse MDR by inhibition of P-gp function in combination with other MDR-reversing drugs using a cytotoxicity assay. *Results*: We established MDR human SCLC cells by culture in medium with gradually increasing concentrations of adriamycin (ADM). Compared with the parental human SCLC cells, SBC-3, the MDR variant SBC-3 cells obtained (SBC-3/ADM) were highly resistant to various chemotherapeutic agents due to P-gp expression. MS-209 reversed the resistance to ADM and vincristine (VCR) of SBC-3/ADM and H69/VP cells in a dose-dependent manner. Moreover, MS-209 in combination with cyclosporin A (CsA) or verapamil (VER) synergistically enhanced the antitumor effects of ADM and VCR on SBC-3/ADM cells. MS-209 restored ADM incorporation and this effect was enhanced by CsA and VER, suggesting that these synergistic effects were due to competitive inhibition of P-gp function. *Conclusion*: MS-209 in combination with CsA or VER might increase the efficacy of these chemotherapeutic agents against MDR human SCLC cells.

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Abbreviations *P-gp* P-glycoprotein · *MDR* multidrug resistance · *SCLC* small cell lung cancer · *ADM* adriamycin · *VCR* vincristine · *CsA* cyclosporin A · *VER* verapamil · *NSCLC* non-small-cell lung cancer · *VP-16* etoposide · *CDDP* cisplatin · *MTT* 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide · IC_{50} concentration for 50% inhibition · *SF* sensitization factor · *MFI* mean fluorescence intensity · *MRP* MDR-associated protein

Introduction

Small-cell lung cancer (SCLC) accounts for 20–25% of all human lung cancers. It differs from other forms of lung cancer, known as non-small-cell lung cancer (NSCLC), in initially being much more responsive to chemotherapy [7, 8]. Up to 90% of SCLC tumors respond to chemotherapy, but patients always relapse, possibly because of development of multidrug resistance (MDR) of the SCLC cells [3]. This possibility is supported by a recent study showing that even a low level of P-gp expression could be useful as a marker of resistance to combination chemotherapy in SCLC as well as ovarian cancer cells [13].

Overexpression of the transmembrane transport protein, P-gp, has been detected in many MDR cancer cell lines and in a variety of tumors of cancer patients with both acquired and inherent drug resistance [11]. This protein is encoded by the human *MDR1* gene, and in vitro studies have shown that it confers drug resistance on cells by acting as a membrane-bound ATP-consuming drug efflux pump [22]. To overcome P-gp-mediated MDR, several classes of compounds capable of inhibiting the P-gp-mediated efflux and so enhancing the accumulation of chemotherapeutic agents have been identified. These include calcium channel blockers [30],

calmodulin inhibitors [9], antiarrhythmics [31], antimalarials [36], steroids [15], antiestrogens [26], reserpine [16], cyclic peptide antibiotics [32], some isoprenoids [23] and cepharanthine [28]. The efficacies of these compounds in animal studies and clinical trials, however, have been disappointing as a result of dose-limiting toxicity. Accordingly, efforts are now being made to identify other compounds that inhibit P-gp function, reverse the MDR phenotype, and sensitize cancer cells to conventional chemotherapy without undesirable toxicological effects.

Recently, a quinoline compound, MS-209, has been shown to be highly effective as an MDR-reversing agent when administered orally at relatively low doses in combination with adriamyim (ADM) or vineristine (VCR) in mice bearing drug-resistant variants of mouse and human leukemia cell lines [1, 27]. Moreover, this drug has been found to have an MDR-reversing effect on blast cells from patients with acute myelogenous leukemia [33]. Administration of MS-209 with ADM has been found to be more effective in potentiating cytotoxic activity against human gastric cancer cells implanted subcutaneously into nude mice [24]. Based on these findings, a clinical phase II study with this new compound is now in progress in Japan.

In this study, we examined the potency of MS-209 in circumventing MDR in MDR human SCLC cells. We also evaluated the effectiveness of combinations of MS-209 and Cyclosporin A (CsA) or Verapamil (VER) in vitro to determine whether the interactions of these compounds would allow the use of a lower concentration of MS-209 clinically for preventing drug resistance.

Materials and methods

Cell lines and cell culture

Human SCLC cells, SBC-3, were kindly provided by Dr. S. Hiraki (Okayama University, Okayama, Japan) [35]. Human SCLC cells, H69 [21], and its etoposide (VP-16)-resistant variant, H69/VP [20], were kindly provided by Dr. N. Saijo (National Cancer Center Research Institute, Tokyo, Japan). Two drug-resistant sublines of SBC-3 were obtained by culturing the cells with gradually increasing concentrations of ADM or cisplatin (CDDP). After 6 months, cells which grew in 100 ng/ml of ADM, and 400 ng/ml of CDDP were obtained and named SBC-3/ADM and SBC-3/CDDP, respectively. Cell cultures were maintained in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, N.Y.) and gentamicin (Schering-Plough, Osaka, Japan) at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cytotoxicity assays were performed when the cultured target cells were in the exponential phase of growth.

Reagents

MS-209 (MW 656 Da) was kindly provided by Mitsui Chemicals (Tokyo, Japan). ADM, VCR, VP-16, CDDP and CPT-11 were purchased from Kyowa Hakko Co. (Tokyo, Japan), Shionogi Pharmaceutical Co. (Osaka, Japan), Nippon Kayaku Co. (Tokyo, Japan), Bristol-Myers Squibb K.K. (Tokyo, Japan) and Daiichi Pharmaceutical Co. (Tokyo, Japan), respectively. CsA (MW 1215 Da) was kindly provided by Sandoz Pharmaceutical Co. (Tokyo, Japan) and VER (MW 491Da) was purchased from Eisai Co. (Tokyo, Japan). The mouse anti-P-gp monoclonal antibody, MRK16 (IgG2a), was obtained as described previously [12].

Analysis of P-gp expression by flow cytometry

Tumor cells were harvested and resuspended in phosphate-buffered Saline (PBS) supplemented with 10% human pooled AB serum to prevent nonspecific antibody binding. After incubation for 30 min at 4 °C, these cells were washed once and incubated for 30 min at 4 °C with 10 μg/ml MRK16 or 10 μg/ml control mouse serum (Tago, Burlingame, Calif.). The cells were washed with PBS, and fluorescein (FITC)-conjugated goat antimouse IgG $F(ab)_2$ (Immunotech S.A., Marseille, France) was added as a second antibody. After 30 min -incubation at 4 °C, they were washed again and the fluorescence intensity was measured with a FACScan (Becton Dickinson, Mountain View, Calif.) [19].

Growth inhibition assay

For in vitro drug experiments, tumor cells (1×10^4 cell) in 100 μl culture medium were seeded into 96-well Microtest III plates (Falcon, Oxford, Calif.). The cells were treated in triplicate with graded concentrations of anticancer agents (50 μl) in the absence or presence of MS-209 (50 μl) and 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) assay was used to measure the cytotoxic effects of the anticancer agents [4]. For evaluation of synergistic effects, growth inhibition assays were done in the presence or absence of fixed concentrations of CsA and VER in combination with different concentrations of MS-209. CsA (0.3 μM) and VER (1 μM) were used at clinically relevant concentrations [14].

The median drug concentration for 50% inhibition (IC_{50}) of tumor cell growth was determined by plotting the logarithm of drug concentration against the growth rate (percentage of control) of treated cells. The sensitization factor (SF) was determined by dividing the IC_{50} for ADM or VCR alone by the IC_{50} in the presence of the indicated chemosensitizer.

Evaluation of ADM incorporation into SCLC cells

ADM has been reported to have intrinsic fluorescence activity [29]. Therefore, we evaluated ADM incorporation into SCLC cells as the mean fluorescence intensity (MFI) of incorporated ADM. To evaluate the effect of MS-209 on the ADM efflux from SCLC cells, we exposed SBC-3 and SBC-3/ADM cells to ADM (30 μg/ml) in the absence or presence of various concentrations of MS-209 at 37 °C for 4 h. After incubation, the cells were harvested and washed twice with ice-cold PBS. Then, the fluorescence intensity of ADM incorporated into the cells was measured with a FACScan. To investigate the effects of MS-209 with CsA or VER on ADM efflux from MDR SCLC cells, we exposed SBC-3/ADM cells to ADM (30 μg/ml) in the presence or absence of a fixed concentration of CsA (0.3 μM) or VER (1 μM) with or without various concentrations of MS-209 at 37 °C for 4 h. After incubation, we measured the fluorescence intensity of ADM incorporated into the cells.

Statistical analysis

To analyze the interactions between MS-209 and CsA or VER, we used the effect multiplication criterion [2]. According to this criterion, the SF of MS-209 with CsA or VER equalled the values for the SF of MS-209 alone plus CsA or VER alone if the drugs did not

Table 1 Evaluation of anticancer drug resistance of human SCLC cells. Tumor cells (1×10^4 cells) were seeded 100 μl culture medium and then treated with graded concentrations of various chemotherapeutic agents (50 μl). After 72 h of continuous drug exposure, the growth-inhibitory effects were evaluated using an MTT assay. Values are means ± SD from four independent experiments

Cell line	IC_{50} (ng/ml)				
	ADM	CDDP	VP-16	VCR	CPT-11
SBC-3	29.94 ± 1.07	185.8 ± 23.8	85.55 ± 8.48	2.12 ± 0.21	344.3 ± 75.7
SBC-3/ADM	1170.9 ± 138*	183.5 ± 24.2	1000.2 ± 186*	1507.3 ± 39.3	536.2 ± 79.9
SBC-3/CDDP	30.42 ± 12.3	770.3 ± 241*	103.4 ± 18.1	1.83 ± 0.15	321.6 ± 41.8

*$P < 0.05$ vs parental SBC-3 cells

interact (additive or so called "expected" effects). If the SF of the combination was higher than expected, the interaction of MS-209 with CsA or VER was synergistic. A value lower than expected indicated a negative interaction (antagonism).

Results

Evaluation of drug-resistance in drug-resistant human SCLC cells

We investigated the degree of drug resistance of established SBC-3/ADM and SBC-3/CDDP cells. As shown in Table 1, SBC-3/ADM cells were much more resistant to ADM (39.1-fold), VP-16 (11.7-fold) and VCR (711.0-fold), but not to CDDP, than the parental SBC-3 cells. SBC-3/CDDP cells were slightly more resistant to CDDP (4.2-fold) than SBC-3 cells.

Analysis of P-gp expression in human SCLC cells

A major cause of MDR is overexpression of P-gp [11, 22]. Therefore, we first examined P-gp expression in MDR human SCLC and their parental cells. Figure 1 shows the reactivities of these SCLC cells to the anti-P-gp monoclonal antibody, MRK16, analyzed by flow cytometry. SBC-3/ADM and H69/VP cells expressed P-gp on their cell surface, whereas SBC-3/CDDP cells did not.

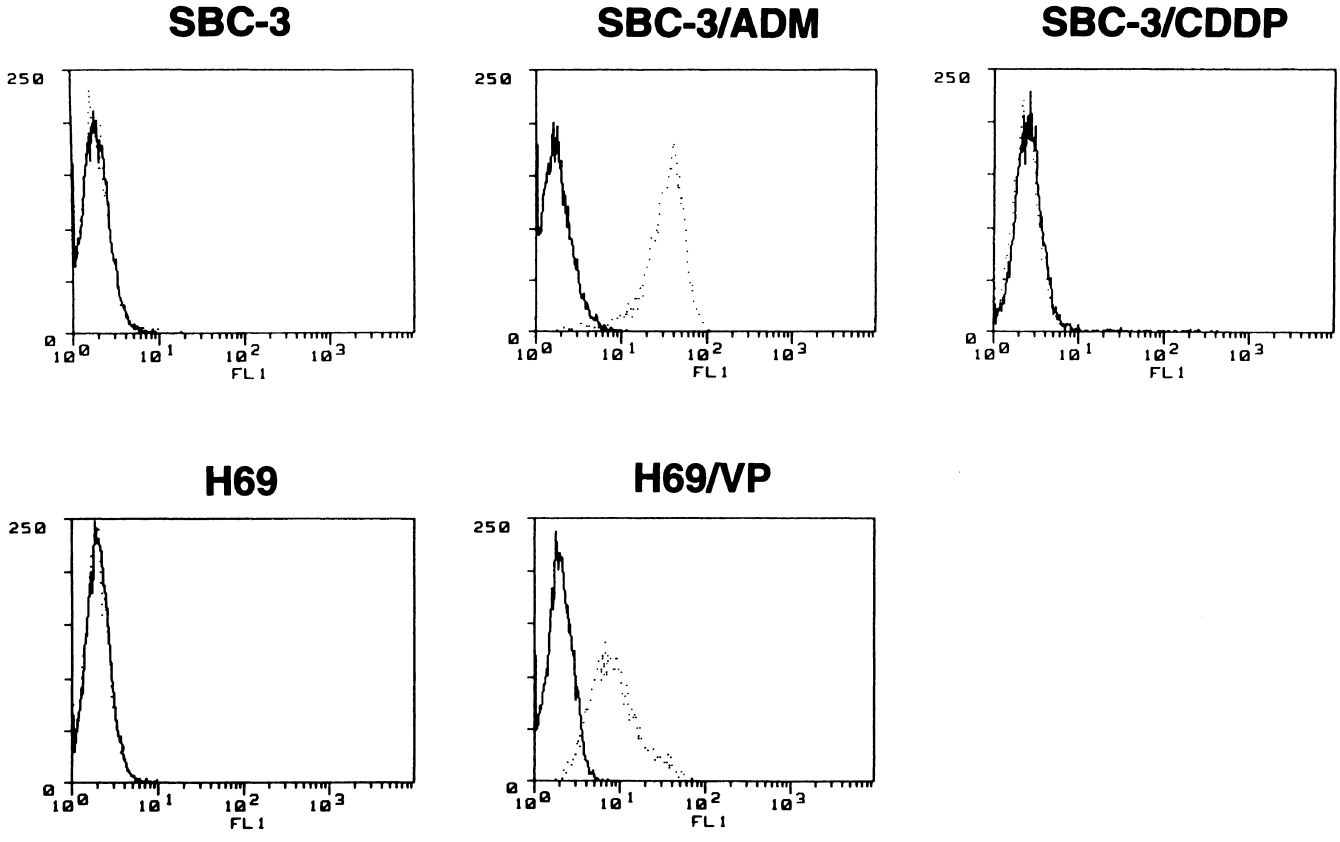

Fig. 1 Expression of P-gp in human SCLC cells. Tumor cells were treated with mouse control serum alone or anti-P-gp monoclonal antibody, MRK16, as described in Materials and methods. Data are representative of two separate experiments

Table 2 Reversal of ADM resistance by MS-209 in MDR human SCLC cells. Tumor cells (1×10^4 cells) were seeded in 100 μl culture medium and then treated with graded concentrations of ADM (50 μl) in the absence or presence of MS-209 (50 μl). After 72 h of continuous drug exposure, the growth-inhibitory effects were evaluated using an MTT assay. Each value is the mean of triplicate determinations. Standard deviations were within 10% of each value. Numbers in parentheses are the values relative to the IC_{50} for each cell line in the absence of MS-209

MS-209 (μ*M*)	IC_{50} (ng/ml) of ADM				
	SBC-3	SBC-3/ADM	SBC-3/CDDP	H69	H69/VP
0	29.9 (1.0)	1170.9 (1.0)	14.3 (1.0)	155.1 (1.0)	1292.6 (1)
0.3	25.0 (1.2)	120.7 (9.7)	10.4 (1.4)	154.7 (1.0)	411.0 (3.1)
1.0	20.2 (1.5)	55.2 (21.2)	10.5 (1.4)	142.9 (1.1)	135.6 (9.5)
3.0	19.9 (1.5)	33.1 (35.4)	9.4 (1.5)	137.3 (1.2)	79.0 (16.4)
10.0	15.9 (1.9)	6.1 (72.7)	8.2 (1.7)	107.6 (1.4)	52.1 (24.1)

Table 3 Reversal of VCR resistance by MS-209 in MDR human SCLC cells. Tumor cells (1×10^4 cells) were seeded in 100 μl culture medium and then treated with graded concentrations of VCR (50 μl) in the absence or presence of MS-209 (50 μl). After 72 h of continuous drug exposure, the growth-inhibitory effects were evaluated using an MTT assay. Each value is the mean of triplicate determinations. Standard deviations were within 10% of each value. Numbers in parentheses are the values relative to the IC_{50} for each cell line in the absence of MS-209

MS-209 (μ*M*)	IC_{50} (ng/ml) of VCR				
	SBC-3	SBC-3/ADM	SBC-3/CDDP	H69	H69/VP
0	2.1 (1.0)	1507.3 (1.0)	1.9 (1.0)	2.2 (1.0)	350.9 (1.0)
0.3	1.6 (1.3)	90.3 (16.7)	1.2 (1.6)	1.9 (1.2)	45.6 (7.7)
1.0	1.5 (1.4)	9.2 (163.8)	1.2 (1.6)	1.9 (1.2)	6.7 (52.4)
3.0	1.2 (1.8)	2.5 (602.9)	0.8 (2.4)	1.7 (1.3)	2.5 (152.6)
10.0	1.1 (2.0)	1.6 (942.1)	0.6 (3.2)	0.9 (2.4)	0.8 (438.6)

Table 4 Synergistic reversal of ADM resistance by MS-209 in combination with CsA or VER in SBC-3/ADM cells. Tumor cells (1×10^4 cells) were seeded in 50 μl culture medium and then treated with graded concentrations of ADM (50 μl) in the absence or presence of MS-209 and either CsA or VER (50 μl respectively). After 72 h of continuous drug exposure, the growth-inhibitory effects were evaluated using an MTT assay. Each value is the mean of triplicate determinations. Standard deviations were within 10% of each value. Numbers in parentheses are the values relative to the IC_{50} for each cell line in the absence of MS-209

Cell line	MS-209 (μ*M*)	IC_{50} (ng/ml) of ADM		
		Medium	CsA (0.3 μ*M*)	VER (1 μ*M*)
SBC-3	0	29.9 (1.0)	23.2 (1.3)	29.0 (1.0)
	0.1	28.6 (1.0)	23.4 (1.3)	28.3 (1.1)
	0.3	25.0 (1.2)	21.1 (1.4)	24.2 (1.2)
	1.0	20.2 (1.5)	19.6 (1.5)	20.2 (1.5)
	3.0	19.9 (1.5)	11.4 (2.6)	19.0 (1.6)
SBC-3/ADM	0	1170.9 (1.0)	225.0 (5.2)	319.3 (3.7)
	0.1	206.2 (5.7)	95.5 (12.3)[a]	95.3 (12.3)[a]
	0.3	120.7 (9.7)	46.9 (25.0)[a]	72.0 (16.3)[a]
	1.0	55.2 (21.2)	32.8 (35.7)[a]	36.3 (32.3)[a]
	3.0	33.1 (35.4)	18.7 (62.6)[a]	20.8 (56.3)[a]

[a] Synergistic effect as determined by the effect multiplication method

Circumvention of ADM and VCR resistance in MDR human SCLC cells

We examined the effect of MS-209 on the proliferation of MDR human SCLC and their parental cells. No significant differences in their proliferation were observed (data not shown).

Next, we examined the potentiating effects of MS-209 on ADM in SBC-3/ADM and H69/VP cells (Table 2). SBC-3/ADM and H69/VP cells showed a 39.1- and 8.3-fold higher resistance, respectively, to ADM than their respective parental cells. The ADM resistances of SBC-3/ADM and H69/VP were completely reversed by MS-209 at concentrations of 1–10 μ*M*. The sensitivities of parental SBC-3 and H69 cells to ADM were also moderately enhanced by MS-209. We also examined the effect of MS-209 on the sensitivities of MDR human SCLC cells and their parental cells to VCR. As shown in Table 3, SBC-3/ADM and H69/VP cells were also much more resistant to VCR than the parental cells (711.0- and 160.0-fold resistance, respectively). The addition of MS-209 at final concentrations of 0.3–10 μ*M* also

Table 5 Synergistic reversal of VCR resistance by MS-209 in combination with CsA or VER in SBC-3/ADM cells. Tumor cells (1×10^4 cells) were seeded in 50 μl culture medium and then treated with graded concentrations of VCR (50 μl) in the absence or presence of MS-209 and either CsA or VER (50 μl, respectively). After 72 h of continuous drug exposure, the growth-inhibitory effects were evaluated using an MTT assay. Each value is the mean of triplicate determinations. Standard deviations were within 10% of each value. Numbers in parentheses are the values relative to the IC_{50} for each cell line in the absence of MS-209

Cell line	MS-209 (μ*M*)	IC_{50} (ng/ml) of VCR		
		Medium	CsA (0.3 μ*M*)	VER (1 μ*M*)
SBC-3	0	2.1 (1.0)	1.3 (1.6)	1.8 (1.2)
	0.1	1.6 (1.3)	1.2 (1.8)	1.8 (1.2)
	0.3	1.6 (1.3)	1.1 (1.9)	1.4 (1.5)
	1.0	1.5 (1.4)	0.9 (2.3)	1.3 (1.6)
	3.0	1.2 (1.8)	1.0 (2.1)	1.2 (1.8)
SBC-3/ADM	0	1507.3 (1.0)	288.6 (5.2)	462.7 (3.3)
	0.1	346.8 (4.3)	103.4 (14.6)[a]	159.5 (9.8)[a]
	0.3	90.3 (16.7)	23.3 (64.7)[a]	47.5 (31.7)[a]
	1.0	9.2 (163.8)	5.2 (289.9)[a]	5.8 (259.8)[a]
	3.0	2.5 (602.9)	1.1 (1370.3)[a]	1.2 (1256.1)[a]

[a] Synergistic effect as determined by the effect multiplication method

reversed the VCR resistance of SBC-3/ADM and H69/VP cells in a dose-dependent manner. The sensitivity of the parental SBC-3 and H69 cells to VCR were also moderately enhanced by MS-209.

Synergistic reversal of MDR by combinations of MS-209 and CsA or VER

The effects of MS-209 with CsA or VER on ADM resistance of SBC-3/ADM cells were investigated (Table 4). Treatment with 0.1, 0.3, 1 and 3 μ*M* MS-209 enhanced the sensitivity of SBC-3/ADM cells to ADM 5.7-, 9.7-, 21.2- and 35.4-fold, respectively. CsA (0.3 μ*M*) alone increased the sensitization 5.2-fold, and synergistically increased the SF of MS-209 12.3-, 25.0-, 35.7- and 62.6-fold at 0.1, 0.3, 1 and 3 μ*M*, respectively, causing complete reversal of ADM resistance at 3 μ*M*. Combinations of MS-209 and VER also had synergistic effects.

Table 5 shows the effects of MS-209 alone and in combination with CsA or VER on the reversal of VCR resistance of SBC-3/ADM cells. The combined effects of MS-209 and CsA (0.3 μ*M*) were more than expected, indicating synergistic reversal of VCR resistance of SBC-3/ADM cells. MS-209 alone at 0.1, 0.3, 1 and 3 μ*M* enhanced the sensitivity of SBC-3/ADM cells to VCR 4.3-, 16.7-, 163.8- and 602.9-fold, respectively. This sensitization was increased 14.6-, 64.7-, 289.9- and 1370.3-fold by 0.3 μM CsA at 0.1, 0.3, 1 and 3 μ*M* of MS-209, respectively. Complete reversal of VCR resistance of SBC-3/ADM cells was achieved at 3 μ*M* MS-209 by CsA, but at 10 μ*M* MS-209 with MS-209 alone (Table 3). The combination of MS-209 and VER (1 μ*M*) also resulted in a synergistic chemosensitizing interaction and was more effective than these compounds individually in reversing VCR resistance. The SF was increased 9.8-, 31.7-, 259.8- and 1256.1-fold with 0.1, 0.3, 1 and 3 μ*M* MS-209, respectively, with complete restoration of VCR sensitivity at 3 μM MS-209. In the parental SBC-3 cells, which do not express P-gp, CsA and VER modulated the ADM and VCR cytotoxicities slightly, but no substantial effects were observed by combinations with MS-209 (Tables 4, 5).

The effect of the combination of MS-209 with ADM was in general less than that with VCR. Nonetheless, combinations of MS-209 with either CsA or VER had synergistic chemosensitizing interactions and were more effective than the individual compounds in reversing ADM resistance.

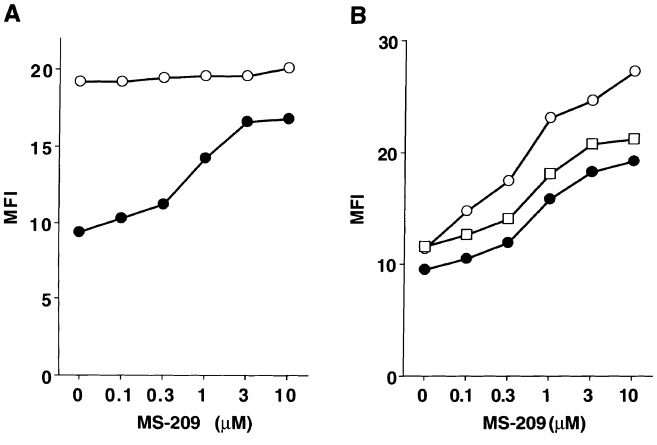

Fig. 2A,B ADM incorporation into SCLC cells. **A** Parental SBC-3 cells (*open circles*) and SBC-3/ADM cells (*closed circles*) were exposed to ADM (30 μg/ml) in the absence or presence of MS-209 at 37 °C for 4 h. After incubation, flow cytometric analysis was performed as described in Materials and methods. Data are representative of three separate experiments. **B** SBC-3/ADM cells were exposed to ADM (30 μg/ml) with or without various concentrations of MS-209 alone (*closed circles*) or with fixed concentrations of CsA (*open circles*) or VER (*open squares*) at 37 °C for 4 h. After incubation, flow cytometric analysis was performed as described in Materials and methods. Data are representative of three separate experiments

Investigation of ADM efflux from human SCLC cells

Finally, we investigated the ADM efflux from human SCLC cells to evaluate the MDR-reversing mechanism of MS-209. The MFIs of ADM incorporated into SBC-3/ADM cells were less than those from the parental SBC-3 cells. However, the addition of MS-209 restored the MFI of ADM incorporated into SBC-3/ADM cells in a dose-dependent manner (Fig. 2A). These results suggest that MS-209 reversed the MDR by direct interaction with P-gp and inhibition of its drug efflux activity. Moreover, the combinations of MS-209 with CsA or VER further restored the MFI of ADM incorporated into SBC-3/ADM cells (Fig. 2B).

Discussion

In the present study, we showed that MS-209 reversed MDR to ADM and VCR in MDR human SBC-3/ADM and H69/VP cells in a dose-dependent manner. Moreover, we demonstrated that combinations of MS-209 with CsA or VER synergistically enhanced the antitumor effects of ADM and VCR against SBC-3/ADM cells. This is the first report of the synergistic effects of MS-209 and other MDR-reversing agents in circumventing MDR.

In this study, we established two cell lines (SBC-3/ADM and SBC-3/CDDP) of human SCLC that were resistant to ADM and CDDP, respectively (Table 1). SBC-3/ADM cells expressed P-gp constitutively on their cell surface, but SBC-3/CDDP cells did not (Fig. 1). These results are consistent with previous reports showing that ADM resistance is related to the expression of P-gp [5] but that CDDP resistance is not [25].

Previous studies have shown that the drug resistance of MDR malignant cells can be overcome by many agents. Recently, the novel quinoline derivative, MS-209, has been reported to inhibit P-gp function and reverse the MDR phenotype of hematopoietic malignant cells [27] and human solid tumor cells such as cancer cell lines of digestive tract origin and ovarian and epidermoid cancer cell lines [1, 24]. This was also observed in the present study using P-gp-positive MDR human SCLC cell lines (SBC-3/ADM and H69/VP), MS-209 causing complete reversal of ADM resistance (Table 2). SBC-3/ADM and H69/VP cells were also highly resistant to VCR, but this resistance was also completely reversed by MS-209 (Table 3). These results confirm and extend previously reported results [1, 27]. Interestingly, we found that MS-209 at 10 μM potentiated the cytotoxicity of ADM and VCR on SBC-3, H69 and SBC-3/CDDP cells (Tables 2, 3), which do not express P-gp. In addition to P-gp, MDR-associated protein (MRP) is also known to be related to the acquisition of MDR [6]. We have confirmed that these cells express MRP (data not shown), suggesting that MS-209 might reverse MRP-mediated MDR. These findings confirm and extend previous findings using different MRP-positive cancer cells [10].

It is likely that the optimal concentrations of many drugs for circumventing P-gp-mediated MDR in tissue culture are well above the maximally tolerated plasma levels. Therefore, clinical trials with MDR-reversing agents are usually associated with undesirable side effects. To overcome this problem, one approach is to use these MDR-reversing agents in combination with a reducion in the dose of each to obtain an additive or synergistic anticancer effect. Indeed, a combination of CsA and VER has previously been shown to reverse VCR resistance in a supraadditive manner in human leukemia cell lines [4, 17]. VER has also been found to interact synergistically with quinine on MDR human multiple myeloma cells [18]. In this study, we also sought to determine whether chemosensitization with MS-209 in combination with CsA or VER was more effective against MDR human SCLC cells than each alone. We found that CsA and VER synergistically potentiated the antitumor effects of MS-209 against ADM- and VCR-resistant cell lines (Tables 4, 5). When used in combination with CsA (0.3 μM) or VER (1 μM), the concentration of MS-209 required to completely reverse ADM and VCR resistance was reduced to 3 μM.

A pharmacokinetic study of CsA has revealed that a peak concentration of 1.2–2.4 μM (1–2 μg/ml) and whole blood levels of 0.2–0.6 μM (0.2–0.5 μg/ml) can be achieved without serious adverse effects [17] and the concentration of VER required for circumvention of MDR in vitro is at least about 2–10 μM (1–5 μg/ml) [17]. MS-209 has been shown to have few side effects [1], but our results suggest that the combined use of MS-209 with CsA (0.3 μM) or VER (1 μM) to circumvent MDR in human SCLC may be clinically safer and more efficient than MS-209 alone. Moreover, we have already shown that CsA may directly enhance the susceptibility of MDR cancer cells to the antibody-dependent cellular cytotoxicity (ADCC) reaction mediated by monocytes [34]. Taken together, these results indicate that the MDR of human cancer cells might be circumvented by a combination of various MDR-reversing agents and other biological response modifiers, such as monoclonal antibodies.

In this study, we demonstrated that MS-209 in combination with CsA or VER had more potency to inhibit the ADM efflux than MS-209 alone (Fig. 2B), suggesting that the effects were due to competitive inhibition of P-gp function. Initial reports suggest that MS-209 has a similar mechanism of action to VER, i.e. competitive binding to the drug binding site on P-gp and then transportation from resistant cells by a mechanism similar to that of antitumor agents [4, 22]. However, the mechanism of the synergistic effect of MDR-reverse demonstrated in this study does not seem to be confined to the competitive inhibition of P-gp function. In addition to the competitive inhibition of P-gp function, there are many possibile effects, such as correction of altered plasma membrane potentials, an allosteric effect on

P-gp, suppression of P-gp production and antigenic modulation of P-gp, that might be induced by MS-209 in combination with CsA or VER. However, as most of these possible mechanisms are not supported by evidence, further investigations are necessary to clarify the exact mechanisms of the synergistic effects.

In summary, MS-209 reversed resistance against ADM and VCR in P-gp-associated MDR human SCLC cells in a dose-dependent manner. Since MS-209 has recently been reported to be therapeutically effective in potentiating the cytotoxicity of an antitumor drug in mice bearing human gastric cancer cells [24], the use of antitumor drugs in combination with MS-209 is expected to be potentially useful for overcoming MDR in human SCLC. Moreover, MS-209 in combination with CsA or VER at suboptimal concentrations synergistically potentiated the anticancer effects of ADM and VCR in MDR human SCLC cells. These results suggest that MS-209 in combination with CsA or VER should be useful in potentiating the efficacy of chemotherapeutic agents against MDR human SCLC cells.

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