

## A New Quinoline Derivative MS-209 Reverses Multidrug Resistance and Inhibits Multiorgan Metastases by P-glycoprotein-expressing Human Small Cell Lung Cancer Cells

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Development of distant metastases and acquired multidrug resistance (MDR) are major problems in therapy for human small cell lung cancer (SCLC). MS-209 is a novel quinoline compound, which reverses P-glycoprotein (P-gp)-mediated MDR. We previously reported that MS-209 reversed *in vitro* MDR of human SCLC (SBC-3/ADM and H69/VP) cells expressing P-gp. In the present study, we determined the therapeutic effect of MS-209 in combination with chemotherapy against multiorgan metastases of MDR SCLC cells. SBC-3/ADM cells expressing P-gp were highly resistant to etoposide (VP-16), adriamycin (ADM), and vincristine (VCR) *in vitro*, compared with parental SBC-3 cells lacking P-gp expression. MS-209 restored chemosensitivity of SBC-3/ADM cells to VP-16, ADM, and VCR in a dose-dependent manner *in vitro*. Intravenous injection with SBC-3 or SBC-3/ADM cells produced metastatic colonies in the liver, kidneys and lymph nodes in natural killer (NK) cell-depleted severe combined immunodeficiency (SCID) mice, though SBC-3/ADM cells more rapidly produced metastases than did SBC-3 cells. Treatment with VP-16 and ADM reduced metastasis formation by SBC-3 cells, whereas the same treatment did not affect metastasis by SBC-3/ADM cells. Although MS-209 alone had no effect on metastasis by SBC-3 or SBC-3/ADM cells, combined use of MS-209 with VP-16 or ADM resulted in marked inhibition of metastasis formation by SBC-3/ADM cells to multiple organs. These findings suggest that MS-209 reversed the MDR of SBC-3/ADM cells, but not SBC-3 cells, growing in the various organs, and inhibited metastasis formation *in vivo*. Therefore, this chemosensitizing agent, MS-209, may be useful for treatment of refractory SCLC patients with multiorgan metastases.

Key words: Multidrug resistance — MS-209 — Small cell lung cancer — Metastasis

Lung cancer is a leading cause of cancer death worldwide, and its incidence is rising particularly steeply in developing countries.<sup>1)</sup> The major problem in the therapy of lung cancer is distant organ metastases. Even at the time of early diagnosis, lung cancer is frequently associated with distant micrometastases. Although small cell lung cancer (SCLC) is sensitive to chemotherapy, it gradually becomes resistant due to the emergence of multidrug-resistant (MDR) cells. Several molecules, such as P-glycoprotein (P-gp),<sup>2)</sup> multidrug resistance-related protein (MRP),<sup>3)</sup> and lung resistant protein (LRP),<sup>4)</sup> have been reported to be associated with MDR. These molecules, located at the plasma membrane, are thought to be ATP-driven efflux pumps for various cytotoxic drugs.<sup>2–4)</sup> These molecules have been detected in several cancer cell lines resistant to various anticancer drugs. Of these molecules, P-gp, encoded by the *mdr1* gene, is the best studied mole-

cule contributing to MDR. Expression of the *mdr1* gene was found to be elevated in intrinsically drug-resistant cancers as well as in some tumors that acquired drug resistance during chemotherapy.<sup>5)</sup> In addition, even a low level of P-gp expression was shown to serve as a marker of resistance to combination chemotherapy in ovarian cancer and small cell lung cancer.<sup>6)</sup> Thus, the selective killing of tumor cells expressing P-gp may be very important for successful cancer therapy.

One strategy for circumvention of the resistance of tumor cells expressing P-gp is combined use of anticancer drugs with chemo-sensitizing agents, such as verapamil,<sup>7)</sup> cyclosporin A,<sup>8)</sup> and non-immunosuppressive PSC-833.<sup>9)</sup> These compounds are thought to bind competitively to P-gp and to inhibit the efflux of anticancer drugs by P-gp.

Recently, a quinoline compound, MS-209, was developed as a new chemo-sensitizing agent for MDR cancer.<sup>10, 11)</sup> It is highly effective when administered orally at relatively low doses in combination with adriamycin (ADM) or vincristine (VCR) in mice bearing drug-resistant variants of mouse and human leukemia cell lines.<sup>10, 11)</sup>

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Moreover, it also reverses MDR of blast cells from patients with acute myelogenous leukemia.<sup>12)</sup> Administration of MS-209 with ADM was found to be more effective in potentiating cytotoxic activity against human gastric cancer cells implanted subcutaneously into nude mice.<sup>13)</sup> Since the critical problem in cancer therapy, especially for SCLC patients, is the formation of metastasis in the distant organs, it is desirable to examine the MDR-reversing effect of MS-209 on multiorgan metastases.

We previously reported that MS-209 reversed the resistance to ADM and VCR in MDR human SCLC (SBC-3/ADM and H69/VP) cells in a dose-dependent manner *in vitro*.<sup>14)</sup> Based on these findings, we examined the therapeutic efficacy of MS-209 combined with anticancer drugs on metastasis to multiple organs by MDR human SCLC cells *in vivo* in the present study.

## MATERIALS AND METHODS

**Reagents** MS-209 (MW: 656) (Fig. 1) was provided by Mitsui Chemicals (Tokyo). Etoposide (VP-16), ADM, VCR and cisplatin (CDDP) were obtained from Nippon Kayaku Co. (Tokyo), Kyowa Hakko Co. (Tokyo), Shionogi Pharmaceutical Co. (Osaka) and Bristol-Myers Squibb K. K. (Tokyo), respectively. Fetal bovine serum (FBS) was purchased from Life Technologies (Rockville, MD). Purified mouse IgG2a was purchased from Chemicon (Temecula, CA). Anti-P-gp monoclonal antibody (mAb) MRK16 (IgG2a) was obtained as described previously.<sup>15)</sup>

**Cell cultures** The human SCLC SBC-3 cells were kindly provided by Dr. S. Hiraki (Okayama University, Okayama).<sup>16)</sup> The drug-resistant subline of SBC-3 (SBC-3/ADM) was established in our department by culturing the cells with gradually increasing concentrations of ADM.<sup>14)</sup> Cell cultures were maintained in Eagle's minimum essential medium (MEM) supplemented with 10% heat-inactivated FBS, 4 mM 2-hydroxyethylpiperazine-N'-2-ethane-

sulfonic acid (HEPES) (Life Technologies) and gentamicin (Schering-Plough, Osaka) at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air. Cytotoxicity assays were performed when the cultured target cells were in the exponential phase of growth.

**Analysis of P-gp expression by flow cytometry** Expression of P-gp was measured by means of flow microfluorimetry as described previously.<sup>17)</sup> Briefly, tumor cells (10<sup>6</sup>) were washed once with phosphate-buffered saline (PBS) supplemented with 2% FBS and 0.05% sodium azide (2% FBS-PBS). The washed cells were incubated for 30 min at 4°C in 2% FBS-PBS with anti-P-gp mAb MRK16 or mouse IgG2a as a negative control. They were then washed twice with 2% FBS-PBS and treated with goat anti-mouse IgG (H+L; Immunotech, Marseille, France) for 30 min at 4°C. The cells were washed again and their fluorescence intensity was measured with a FACScan (Becton Dickinson, Mountain View, CA).

***In vitro* proliferation and chemosensitivity assays** Cell proliferation was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction method.<sup>18)</sup> Briefly, 1×10<sup>4</sup> cells were plated into each well of 96-well plates and treated with various concentrations of drugs. After a 72-h incubation at 37°C, 50 µl of the MTT stock solution (5 mg/ml) was added to each well, and the cells were further incubated for 2 h at 37°C. Then, the culture media were removed, and 100 µl of dimethylsulfoxide was added to dissolve the dark blue crystals. Absorbances were measured with an MTP-32 Microplate Reader (Corona Electric, Ibaraki) at test and reference wavelengths of 550 and 630 nm, respectively.

The median drug concentration for 50% inhibition (IC<sub>50</sub>) of tumor cell-growth was determined by plotting the logarithm of the drug concentration against the growth rate (percentage of control) of treated cells. The sensitization factor (SF) was determined by dividing the IC<sub>50</sub> for VP-16, ADM, or VCR alone by the IC<sub>50</sub> in the presence of MS-209.

**Animals** Male severe combined immunodeficiency (SCID) mice, 6–8 weeks of age, were obtained from Charles River (Yokohama) and maintained under specific pathogen-free conditions. Experiments were performed according to the guidelines of our university (University of Tokushima).

**Experimental metastasis** For experimental production of metastases of human lung cancer cells, SCID mice were injected i.p. with TM-β1 Ab (1 mg/mouse) 2 days before tumor inoculation to deplete natural killer (NK) cells as described previously.<sup>19)</sup> Then, viable tumor (SBC-3 or SBC-3/ADM) cells suspended in 300 µl of PBS were injected into the lateral tail vein. The mice inoculated with SBC-3 cells were treated with oral feeding with vehicle or MS-209 and i.v. injection with saline, VP-16 or ADM on days 14, 15, 21, and 22. The mice inoculated with SBC-3/

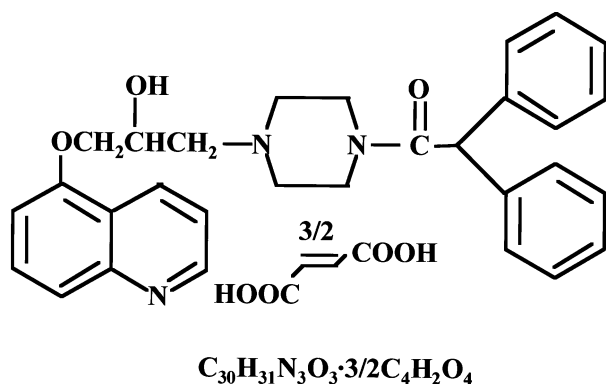


Fig. 1. Chemical structure of MS-209.

ADM cells received the same treatment on days 10, 11, 17, and 18. After the indicated periods, the mice were sacrificed, and the numbers of metastatic lymph nodes were counted. Nodules longer than 0.5 mm diameter in the liver and kidneys were counted with the aid of a dissecting microscope. For therapy experiments, VP-16, ADM, or VCR was injected i.v. and MS-209, which was suspended in distilled water containing 0.5% Tween 80 (Sigma Chemical Co., St. Louis, MO), was administered perorally (p.o) on various days after tumor inoculation.

**Statistical analysis** The significance of differences of *in vitro* findings was analyzed by using Student's *t* test (two-tailed) and the significance of differences of *in vivo* findings was analyzed by means of the Mann-Whitney *U* test.

## RESULTS

**P-gp expression and chemosensitivity of SBC-3 and SBC-3/ADM cells *in vitro*** In the first set of experiments, we evaluated P-gp expression and drug sensitivity in human SCLC SBC-3 cells and its MDR variant, SBC-3/ADM cells *in vitro*. To clarify P-gp expression on SBC-3

and SBC-3/ADM cells, the reactivity of these SCLC cells to anti-P-gp mAb (MRK16) was analyzed by flow cytometry. As shown in Table I, SBC-3/ADM cells expressed P-gp on the cell surface, whereas SBC-3 cells did not. SBC-3/ADM cells were much more resistant to VP-16, ADM and VCR than SBC-3 cells. SBC-3/ADM cells were as sensitive to CDDP as SBC-3 cells. The *in vitro* doubling time of SBC-3/ADM cells was shorter than that of SBC-3 cells.

**Chemosensitizing effect of MS-209 against MDR human SCLC SBC-3/ADM cells *in vitro*** We next examined the chemosensitizing effect of MS-209 against SBC-3 and SBC-3/ADM cells, in the presence of VP-16, ADM, or VCR (Table II). SBC-3/ADM cells were much more resistant to VP-16, ADM and VCR than SBC-3 cells (10.6-, 44.2- and 734.6-fold resistance, respectively). The multidrug resistance of SBC-3/ADM cells was completely reversed by MS-209 at concentrations of 3–10  $\mu$ M. The sensitivity of SBC-3 to VP-16, ADM and VCR were only marginally enhanced by MS-209. We also examined the effect of MS-209 on the proliferation of SBC-3 and SBC-3/ADM cells. Using up to 30  $\mu$ M concentrations, MS-209

Table I. *In vitro* Characterization of SBC-3 and SBC-3/ADM Cells

Cell line	P-gp expression (control) <sup>a)</sup>	IC <sub>50</sub> <sup>b)</sup> (ng/ml)				<i>In vitro</i> proliferation <sup>b)</sup> (doubling time, h)
		VP-16	ADM	VCR	CDDP	
SBC-3	2.95 (2.84)	107.7±13.9	21.7±4.1	1.7±0.1	168.8±18.9	22.1±2.6
SBC-3/ADM	150.33 (2.95)	1136.3±203.5	956.0±167.2	1263.5±95.2	162.5±23.1	16.9±1.9

a) Surface antigen expression was measured by flow cytometry. Values are expressed as mean fluorescence.

b) Drug sensitivity and *in vitro* proliferation of tumor cells were measured by MTT assay.

Table II. Reversal of VP-16, ADM, or VCR by MS-209 in MDR Human SCLC SBC-3/ADM Cells *in vitro*

Cell line	MS-209 ( $\mu$ M)	IC <sub>50</sub> <sup>a)</sup> (ng/ml)		
		VP-16	ADM	VCR
SBC-3	0	110.2 (1.0)	23.8 (1.0)	1.7 (1.0)
	0.3	100.9 (1.1)	20.4 (1.2)	1.4 (1.2)
	1.0	91.3 (1.2)	18.8 (1.3)	1.2 (1.4)
	3.0	75.5 (1.5)	15.4 (1.5)	1.0 (1.7)
	10.0	66.3 (1.7)	13.6 (1.8)	0.9 (1.9)
SBC-3/ADM	0	1208.3 (1.0)	922.6 (1.0)	1324.9 (1.0)
	0.3	348.3 (3.5)	105.9 (8.7)	62.7 (21.1)
	1.0	256.8 (4.7)	43.1 (21.4)	5.5 (240.9)
	3.0	122.6 (9.9)	27.9 (33.1)	1.9 (697.3)
	10.0	69.3 (17.4)	8.8 (104.8)	0.9 (1472.1)

a) Drug sensitivity was measured by MTT assay. Numbers in parentheses are the values relative to the IC<sub>50</sub> for each cell line in the absence of MS-209.

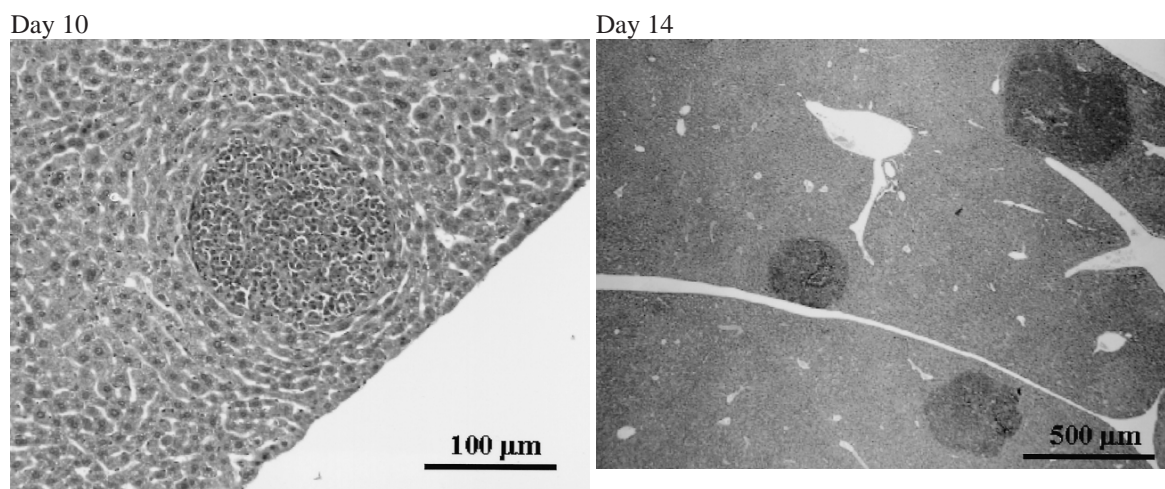


Fig. 2. Micrometastasis of SBC-3/ADM cells in NK cell-depleted SCID mice. NK cell-depleted SCID mice were injected i.v. with SBC-3/ADM ( $1 \times 10^6$ ) cells. The mice were sacrificed and autopsied 10 days later. All major organs were fixed in 10% phosphate-buffered formalin, and sections were cut and stained with hematoxylin and eosin using standard procedures. Micrometastases in the liver are shown.

Table III. Effect of MS-209 on the Antimetastatic Activity of VP-16 and ADM in Human SCLC SBC-3 Cell-bearing NK Cell-depleted SCID Mice<sup>a)</sup>

Treatment	Number of metastatic colonies (mean±SD)			Liver weight (g)
	Liver	Kidneys	Lymph nodes	
Experiment 1				
Vehicle and saline	16±3	10±2	11±4	5.4±0.9
MS-209 and saline	18±4	11±2	12±5	5.6±0.6
Vehicle and VP-16	10±3 <sup>b)</sup>	5±2 <sup>b)</sup>	8±2	2.0±0.3 <sup>b)</sup>
MS-209 and VP-16	8±3	5±3	6±4	1.9±0.3
Experiment 2				
Vehicle and saline	18±5	10±3	13±3	4.8±0.5
MS-209 and saline	18±5	11±2	13±3	4.9±0.3
Vehicle and VP-16	11±3 <sup>b)</sup>	6±2 <sup>b)</sup>	8±2 <sup>b)</sup>	2.2±0.3 <sup>b)</sup>
MS-209 and VP-16	9±2	6±2	8±3	1.9±0.2
Vehicle and ADM	14±4	7±2	9±2	2.7±0.5 <sup>b)</sup>
MS-209 and ADM	13±4	8±2	9±1	2.8±0.6

a) NK cell-depleted SCID mice were injected i.v. with human SCLC SBC-3 ( $1 \times 10^6$ ) cells on day 0. Vehicle (0.5% Tween 80) or MS-209 (200 mg/kg) was administered p.o. and saline (0.5 ml), VP-16 (4 mg/kg), or ADM (2 mg/kg) was administered i.v. twice per week from day 14 (on days 14, 15, 21, and 22). All mice ( $n=5$ ) were sacrificed on day 42. Metastatic colonies were counted as described in "Materials and Methods."

b)  $P < 0.05$  vs. value for control.

did not affect the proliferation of these two cell lines *in vitro* (data not shown).

**Antimetastatic effect of MS-209 in combination with anticancer drugs in NK cell-depleted SCID mice** We previously reported that NK cell depletion in SCID mice facilitates the metastasis formation of human lung cancer cell lines.<sup>19)</sup> In NK cell-depleted SCID mice, SBC-3 cells inoculated i.v. produced micrometastases in the liver and

kidneys by day 14, and the recipient mice became moribund by day 42, as reported previously.<sup>20)</sup> However, SBC-3/ADM cells inoculated i.v. developed micrometastases in the liver by day 10 (Fig. 2), and the recipient mice became moribund by day 28, indicating a more aggressive phenotype of SBC-3/ADM cells compared with SBC-3 cells *in vivo*. Using these models, we investigated the antimetastatic effect of MS-209 in combination with chemotherapy.

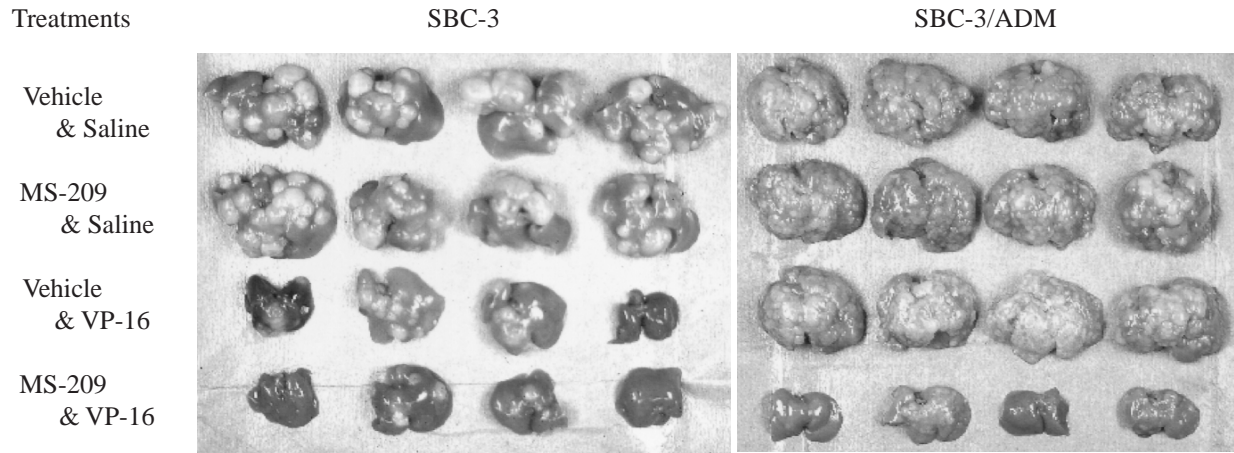


Fig. 3. Therapeutic efficacy of MS-209 in combination with chemotherapy on liver metastasis by SBC-3 or SBC-3/ADM cells. (A) NK cell-depleted SCID mice were injected i.v. with human SCLC SBC-3 ( $1 \times 10^6$ ) cells on day 0. Vehicle (0.5% Tween 80) or MS-209 (200 mg/kg) was administered p.o. and saline (0.5 ml) or VP-16 (4 mg/kg) was administered i.v. on days 14, 15, 21, and 22. All mice ( $n=5$ ) were sacrificed on day 42. (B) NK cell-depleted SCID mice were injected i.v. with MDR human SCLC SBC-3/ADM ( $1 \times 10^6$ ) cells on day 0. Vehicle (0.5% Tween 80) or MS-209 (200 mg/kg) was administered p.o. and saline (0.5 ml) or VP-16 (4 mg/kg) was administered i.v. on days 10, 11, 17, and 18. All mice ( $n=5$ ) were sacrificed on day 28.

Table IV. Effect of MS-209 on the Antimetastatic Activity of VP-16 and ADM in MDR Human SCLC SBC-3/ADM Cells-bearing NK Cell-depleted SCID Mice<sup>a)</sup>

Treatment	Number of metastatic colonies (mean±SD)			Liver weight (g)
	Liver	Kidneys	Lymph nodes	
Experiment 1				
Vehicle and saline	78±11	5±2	10±2	7.2±0.9
MS-209 and saline	81±15	5±2	11±3	8.0±1.1
Vehicle and VP-16	76±12	4±1	10±3	7.7±1.1
MS-209 and VP-16	32±20 <sup>b)</sup>	1±1 <sup>b)</sup>	3±2 <sup>b)</sup>	2.2±0.6 <sup>b)</sup>
Experiment 2				
Vehicle and saline	64±14	4±1	8±2	6.9±0.7
MS-209 and saline	65±14	5±1	10±3	7.1±1.2
Vehicle and VP-16	61±12	4±1	9±2	6.8±1.0
MS-209 and VP-16	31±13 <sup>b)</sup>	1±1 <sup>b)</sup>	5±1 <sup>b)</sup>	2.8±0.6 <sup>b)</sup>
Vehicle and ADM	63±10	4±1	8±2	6.9±1.0
MS-209 and ADM	40±12 <sup>b)</sup>	2±1	5±1 <sup>b)</sup>	4.0±1.1 <sup>b)</sup>

a) NK cell-depleted SCID mice were injected i.v. with MDR human SCLC SBC-3/ADM ( $1 \times 10^6$ ) cells on day 0. Vehicle (0.5% Tween 80) or MS-209 (200 mg/kg) was administered p.o. and saline (0.5 ml), VP-16 (4 mg/kg), or ADM (2 mg/kg) was administered i.v. twice per week from day 10 (on days 10, 11, 17, and 18). All mice ( $n=5$ ) were sacrificed on day 28. Metastatic colonies were counted as described in "Materials and Methods."

b)  $P < 0.05$  vs. value for control.

To clarify the effect of MS-209 against established metastases, the mice inoculated with SBC-3 were given the treatment with MS-209 and anticancer agents on days 14, 15, 21 and 22, and were sacrificed on day 42. The mice inoculated with SBC-3/ADM cells were given the

same treatment on days 10, 11, 17 and 18, and were sacrificed on day 28.

As shown in Table III, SBC-3 cells inoculated i.v. developed metastatic colonies in the liver, kidneys and lymph nodes, and treatment with MS-209 alone had no

effect on these metastases. Treatment with VP-16 alone or ADM alone reduced metastasis formation by SBC-3 cells, as well as the liver weight, which represents the volume of liver lesions. However, further reduction of metastasis formation was not obtained by addition of MS-209 (Fig. 3).

SBC-3/ADM cells inoculated i.v. produced metastases to multiple organs more rapidly than did SBC-3 cells, whereas the pattern of metastasis (the liver, kidneys, and systemic lymph nodes) produced by these two cell lines was identical. As shown in Table IV, neither MS-209 alone nor anticancer drug alone inhibited metastasis formation by SBC-3/ADM cells, indicating MDR of SBC-3/ADM cells even *in vivo*. However, treatment with MS-209 in combination with VP-16 or ADM markedly inhibited metastasis formation by SBC-3/ADM cells to the liver, kidneys and lymph nodes (Fig. 3). Consistent with the reduction of the number of liver metastases, the weight of the liver of the mice treated with MS-209 and anticancer drugs was significantly less than that of other groups (Table IV).

## DISCUSSION

Since metastasis to multiple organs and acquired MDR are major obstacles to treatment for SCLC patients, it is necessary to establish a novel therapeutic modality against metastases of MDR SCLC cells. Several lines of evidence indicate that the effect of antimetastatic agents can be differentially modulated by organ microenvironments,<sup>21, 22)</sup> because of, in part, a different host cell population and different pharmacokinetics. Therefore, it is important to explore whether MS-209 reverses the MDR of metastatic SCLC cells in various organs. We showed that oral feeding with MS-209 in combination with anticancer drugs inhibited the formation of multiorgan metastases by SBC-3/ADM cells expressing P-gp. Since treatment with MS-209 and anticancer drugs started after the development of micrometastases, this treatment inhibited the growth of metastatic MDR-tumor cells in the organs. MS-209 has been reported to bind competitively to the drug-binding site on P-gp and to inhibit efflux of the drug from MDR cells. In addition, we have demonstrated that MS-209 augmented incorporation of ADM into SBC-3/ADM cells, but not parental SBC-3 cells, in a dose-dependent manner.<sup>14)</sup> Collectively, these findings suggest that orally administered MS-209 was distributed to various organs, blocked the function of P-gp expressed on SBC-3/ADM cells, augmented the intracellular concentration of anticancer drugs, and overcame the MDR of SBC-3/ADM cells growing in the various organs.

To clarify the therapeutic effect of MS-209 on metastasis, the drug-sensitive SCLC cell line (SBC-3) and its MDR variant (SBC-3) expressing P-gp were used in the present study. Interestingly, SBC-3/ADM cells grew faster

than SBC-3 cells *in vitro*. Moreover, SBC-3/ADM cells produced metastases into multiple organs more aggressively than SBC-3 cells, although there was no difference in the pattern of metastasis produced by these two cell lines. Previous studies showed that drug sensitivity did not correlate with the metastatic potential of tumor cells.<sup>23)</sup> Indeed, there was no difference in metastatic potential between the parental SCLC cell line, H69, and its MDR variant, H69/VP, expressing P-gp even in our metastasis model.<sup>24)</sup> Therefore, the enhanced metastatic potential of SBC-3/ADM cells may be due to, at least in part, increased cell proliferation, but not MDR phenotype. MS-209, even in combination with anticancer drugs, did not affect metastases produced by SBC-3 cells. These findings suggest that MS-209 restores the chemosensitivity of MDR tumor cells, but does not affect the metastatic potential.

P-gp is physiologically expressed in various organs, including the blood brain barrier, lung, hematopoietic progenitor cells, liver, pancreas, and kidneys.<sup>2)</sup> A previous study reported that MS-209 actually increased ADM accumulation in kidney, liver, lung, heart, and plasma.<sup>13)</sup> Therefore, it is possible that MS-209 increases the toxicity of anticancer drugs to normal cells expressing P-gp. When higher doses of anticancer drugs were used with MS-209, treatment-associated death was observed (data not shown). However, the combined therapy of MS-209 with ADM or VP-16 performed in the present study is feasible.

Another strategy to eradicate micrometastasis produced by P-gp-expressing cancer cells is immunotherapy with anti-P-gp antibodies. We reported that two mouse monoclonal antibodies, MRK16 and MRK17, reacting with the extracellular domain of P-gp, induced lysis of MDR cancer cells by inducing antibody-dependent cellular cytotoxicity by human effector cells (monocyte-macrophages and lymphocytes), and reduced metastasis by MDR SCLC cells in SCID mouse models.<sup>24)</sup> In addition, the antimetastatic efficacy of MRK16 was further enhanced by monocyte chemoattractant protein-1 (*MCP-1*)-gene or macrophage colony-stimulating factor (*M-CSF*)-gene transfection to augment accumulation and cytotoxic activity of monocyte-macrophages.<sup>17, 24)</sup> Based on these findings together with those of present study, multimodality therapy consisting of chemotherapy and MS-209 in combination with these immunotherapies might be effective for circumvention of metastasis of MDR SCLC cells.

In summary, a quinoline derivative, MS-209, combined with chemotherapy reversed the MDR of P-gp-expressing SCLC cells and inhibited their metastasis to multiple organs, such as the liver, kidneys, and systemic lymph nodes. Therefore, MS-209 combined with chemotherapy may be useful for treatment of MDR SCLC with multiorgan metastases.

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