Cancer Chemother Pharmacol (1997) 40 (Suppl): S20–S24

ORIGINAL ARTICLE

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New multidrug-resistance-reversing drugs, MS-209 and SDZ PSC 833

Abstract The emergence of multidrug resistance (MDR) is a major problem in cancer chemotherapy. Many compounds have been developed to reverse MDR, and some of them are undergoing clinical trials. Among them, MS-209, a novel quinoline derivative, is one of the most potent MDR-reversing agents: MS-209 at 3 μ*M* effectively reverses MDR in various cell lines in vitro. MS-209 directly interacts with P-glycoprotein (Pgp) and inhibits Pgp-mediated drug transport. Oral administration of MS-209 combined with anticancer drugs significantly increases the life span of mice bearing MDR tumor cells without causing serious side effects. SDZ PSC 833, a non-immunosuppressive analogue of cyclosporin A (CsA), is another potent MDR-reversing drug. Interestingly, the MDR-reversing activity of SDZ PSC 833 is enhanced in vitro and in vivo by MRK-16, a monoclonal antibody that recognizes an extracellular epitope of Pgp. Since MRK-16 promotes immune responses to MDR tumor cells expressing Pgp, the combined use of MRK-16, SDZ PSC 833, and antitumor drugs could be an effective therapeutic modality to reverse MDR.

Key words MDR reversal · MS-209 · SDZ PSC 833 · MRK-16

Work presented at the 12th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "New therapeutic strategies for higher cure rates: High-dose therapy and new therapeutic modalities," 4–5 October 1996, Nagoya, Japan

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Introduction

Drug resistance is a major obstacle to successful cancer chemotherapy. When tumor cells acquire resistance against one chemotherapeutic drug, they often show cross-resistance to a variety of antitumor drugs. The mechanism of multidrug resistance (MDR) has been well studied, and P-glycoprotein (Pgp), an efflux pump for hydrophobic antitumor drugs, has been shown to play a key role in MDR [6, 12, 19]. A number of compounds, such as verapamil [20, 21] and the cyclosporins [16, 17, 23], have been reported to reverse MDR in vitro and in vivo when combined with antitumor drugs. Although these drugs have MDR-reversing effects in patients with MDR tumors [5, 9, 18], their side effects limit their clinical application. Therefore, new MDR-reversing drugs without serious side effects are needed for clinical reversal of MDR. In this paper we describe two potent MDR-reversing agents, MS-209 [2, 14] and SDZ PSC 833 (Fig. 1) [1, 3, 4, 7, 8, 11, 24, 25].

Materials and methods

Cell culture and drug treatment

Tumor cells were cultured in growth medium (RPMI 1640 medium containing 5% fetal bovine serum and kanamycin at 100 μg/ml) in a humidified atmosphere comprising 5% CO_2 and 95% air. For the drug treatment experiments, 4×10^4 cells were cultured at 37 °C for 5 h in 2 ml of growth medium. Different drug concentrations were then applied, and the cells were reincubated for 72 h in the presence of the drugs and then counted using a model ZBI Coulter counter (Coulter Electronics, Hialeah, Fla., USA), as described previously [20]. Three samples were used for each drug concentration. In control cultures, tumor cells grew exponentially during the incubation period.

When the effect of MDR-reversing drugs was examined, the test drug was added to the culture before the antitumor agents and the cells were counted as described above. The drug concentration necessary to inhibit tumor cell growth by 50% (IC_{50}) was determined by plotting the logarithm of the drug concentration against the growth rate (percentage of control) of the treated cells.

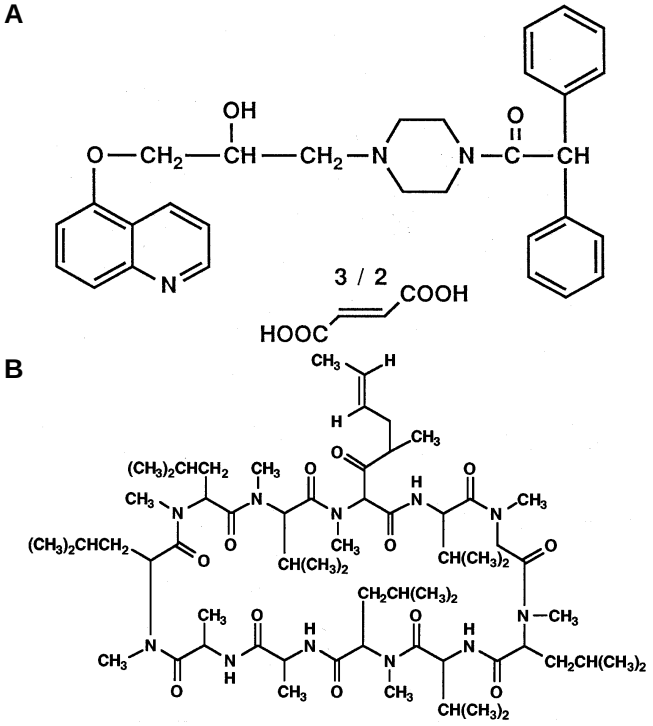

Fig. 1A, B Structure of **A** MS-209 and **B** SDZ PSC 833

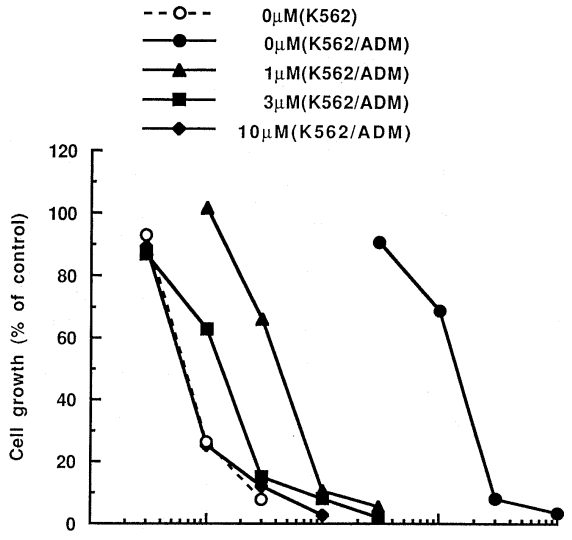

Fig. 2 Enhancement of ADM cytotoxicity for K562/ADM cells by MS-209. K562 cells (○) or K562/ADM cells (◆, ■, ▲, ●) were cultured for 4 days in the presence of the indicated concentrations of ADM in the absence (○, ●) or presence of 1 μ*M* (▲), 3 μ*M* (■), or 10 μ*M* (◆) MS-209

Drug accumulation and efflux

K562/ADM cells were incubated with isotope-labeled drugs for 2 h at 37 °C. Cells were then washed with ice-cold phosphate-buffered saline and intracellular drug accumulation was determined as described previously [21, 22]. Drug efflux was examined essentially as described previously [22].

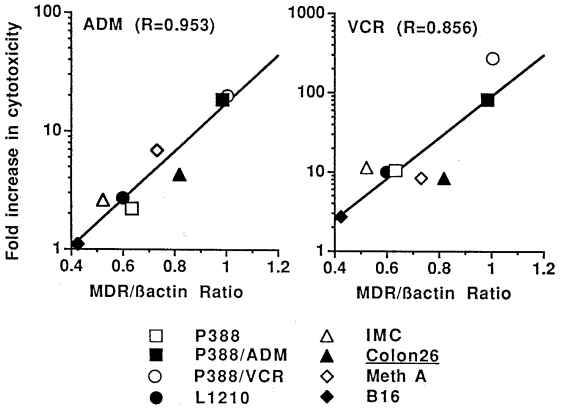

Fig. 3A, B Correlation between MDR gene expression and the MDR-reversing activity of MS-209 in various cell lines (□ P388; ■ P388/ADM; ○ P388/VCR; ● L1210; △ IMC; ▲ colon 26; ◇ Meth A; ◆ B16). Expression of the MDR gene and the β-actin gene, as a reference, was examined by quantitative reverse transcriptase-polymerase chain reaction analysis, and the expression ratio MDR/β-actin was compared with the order of magnitude of increase in the cytotoxicity of **A** ADM and **B** VCR

Evaluation of antitumor activity

P388/ADM cells (10^6 cells/mouse) were transplanted into the peritoneum of CD2F1 mice. Various doxorubicin (ADM) doses were injected intraperitoneally for 5 consecutive days, starting on the day following tumor inoculation, and MS-209 was given orally once daily. Six mice were used in each experimental group. Antitumor activity was evaluated based on the median survival time of the experimental group and was expressed as the treated/control (T/C) value.

Results and discussion

MS-209

MS-209 is a newly synthesized quinoline derivative. Figure 2 shows the MDR-reversal activity of MS-209 on the ADM resistance of K562/ADM cells in vitro; the resistance of K562/ADM cells to ADM is approximately 300-fold that of parental K562 cells. When 1, 3, or 10 μ*M* MS-209 was added to the culture the sensitivity of the K562/ADM cells to ADM was enhanced, and complete reversal of resistance was attained with 10 μ*M* MS-209.

MS-209 was also effective in reversing resistance to various MDR-related drugs. P388/VCR cells show resistance to drugs such as vincristine (VCR), ADM, daunorubicin, and epirubicin. Combined treatment with 3 μ*M* MS-209 reduced the IC_{50} values of the drugs in P388/VCR cells to values lower than those obtained in parental P388 cells not treated with MS-209, indicating that MS-209 is a potent MDR-reversing agent in vitro. MS-209 also enhanced the sensitivity of parental P388 cells to some drugs by several orders of magnitude, and the increase in the cytotoxicity of VCR and ADM in the various cell lines tested depended on the amount of Pgp expressed. Figure 3 shows that the more Pgp expressed by the cells, the more

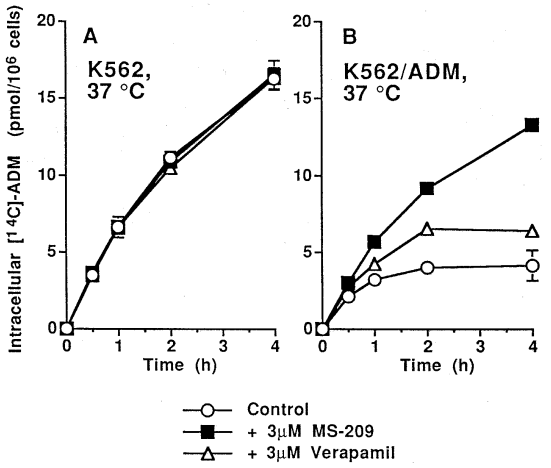

Fig. 4A, B Effect of MS-209 on the accumulation of ADM in K562/ADM cells. **A** K562 and **B** K562/ADM cells were incubated with 50 n*M* ADM alone (○) or in the presence of 3 μ*M* MS-209 (■) or 3 μ*M* verapamil (△)

MS-209 sensitized the cells to VCR and ADM. This result suggests that MS-209 interacts with Pgp.

We next carried out a mechanistic analysis of MS-209. K562/ADM cells accumulated small amounts of ADM, whereas parental K562 cells accumulated a large amount in a time-dependent manner. When K562/ADM cells were treated with 3 μ*M* MS-209, ADM accumulation was increased to amounts comparable to that seen in parental K562 cells (Fig. 4). MS-209 also showed efficient inhibition of active ADM efflux from K562/ADM cells. Most MDR-reversing drugs, such as verapamil and cyclosporins, directly interact with Pgp [26]. MS-209 also directly interacts with Pgp as indicated by its effective inhibition of azidopine photoaffinity labeling of Pgp [14].

We subsequently examined the resistance-reversing activity of MS-209 in vivo. Table 1 shows a representative result obtained using P388/ADM-bearing mice. P388/ADM cells are strongly resistant to ADM; therefore, most resistance-reversing agents have not shown satisfactory effects in therapeutic experiments with P388/ADM-bearing mice. However, oral administration of 300 mg/kg MS-209 combined with 2 mg/kg ADM produced a T/C value of 194%. This result indicates that MS-209 is an orally active and very potent MDR-reversing drug.

We also assessed the therapeutic effect of MS-209 in a solid tumor model. Colon 26 cells were inoculated subcutaneously into mice on day 0 and intravenous ADM and oral MS-209 were given on days 1, 5, and 9. When mice were not treated, the tumors developed in a time-dependent manner. Treatment with 8 mg/kg ADM significantly reduced tumor development, but when this ADM dose was given in combination with MS-209 tumor volume was significantly reduced. Moreover, two or three of six mice were cured by this treatment. When ADM was combined with verapamil, suppression of tumor volume and some cures were observed, but 1 or 3 of 6 mice died due to the cardiotoxic effect of verapamil.

Table 1 Effect of MS-209 on the antitumor activity of ADM in P388/ADM cell-bearing mice

Treatment	Number of mice	Median survival (days)	T/C (%)
Control	12	9.0	100
2 mg/kg ADM	6	11.0	122
+ 200 mg/kg MS-209	6	13.5	150
+ 300 mg/kg MS-209	6	17.5	194
+ 450 mg/kg MS-209	6	15.5	172

Table 2 Potentiation of VCR cytotoxicity for K562/ADM cells by MRK-16[a]

Modifier	Concentration (μM)	IC_{50} of VCR (n*M*)	
		No MRK-16	Plus MRK-16
PSC 833	0	735 (1.0)[b]	509 (1.4)
	1	271 (2.7)	24 (31)[c]
	3	7.4 (99)	2.0 (368)[c]
	10	1.7 (432)	1.8 (408)
CsA	0	639 (1.0)	440 (1.5)
	1	460 (1.4)	99 (6.5)[c]
	3	155 (4.1)	7.7 (83)[c]
	10	5.2 (123)	1.9 (336)[c]

[a] Cells were treated with different concentrations of VCR in the presence or absence of the indicated concentrations of SDZ PSC 833 and CsA with or without MRK-16 at 10 mg/ml. The IC_{50} values of VCR were then determined

[b] Numbers in parentheses indicate the order of magnitude of decreases in the IC_{50} values as compared to the values obtained in the absence of modifier and MRK-16

[c] Synergistic as determined by the effect multiplication method

We also investigated the effect of MS-209 on the myelotoxicity of ADM. ADM at 10 or 20 mg/kg reduced the number of marrow cells seen at 3 days after administration. MS-209 slightly reduced the cell number at day 3 but had a negligible effect on the recovery of marrow cells. In addition, the blood MS-209 concentration required for effective MDR reversal was attained for more than 12 h without producing serious symptoms that might have limited the dose delivered. Thus, MS-209 is an orally active MDR-reversing drug without serious toxicity. MS-209 is now undergoing clinical trials in Japan.

SDZ PSC 833

SDZ PSC 833 is another potent MDR-reversing drug and is an analogue of CsA that has no immunosuppressive activity. Both SDZ PSC 833 and CsA reverse MDR efficiently. As shown in Table 2, in K562/ADM cells, 99- and 432-fold sensitizations were attained using 3 and 10 μ*M* SDZ PSC 833, respectively, and 123-fold sensitization was obtained using 10 μ*M* CsA. Therefore, SDZ PSC 833 is more effective than CsA at a lower concentration.

Interestingly, the resistance-reversing activity of CsA and SDZ PSC 833 was enhanced when MRK-16, a monoclonal antibody that recognizes the external epitope of Pgp, was also used, whereas MRK-16 alone showed only a

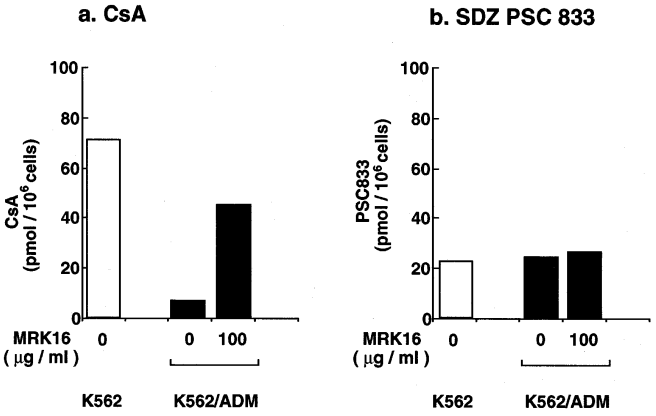

Fig. 5A, B Effect of MRK-16 on the intracellular accumulation of SDZ PSC 833 and CsA. Cells were incubated with **A** [^{3}H]-CsA or **B** [^{14}C]-SDZ PSC 833 in the presence of the indicated concentrations of MRK-16

minimal effect. MRK-16 enhances cellular accumulation of antitumor drugs given in combination with suboptimal doses of SDZ PSC 833 and CsA [10, 11].

CsA is a good substrate for Pgp-mediated transport [13, 15]; therefore, CsA accumulation in resistant cells was very low compared to that observed in parental cells (Fig. 5), although MRK-16 increased CsA accumulation in resistant cells. This may account for the potentiation of the resistance-reversing activity of CsA by MRK-16. However, the accumulation of SDZ PSC 833 in resistant cells was comparable to that seen in parental cells, and MRK-16 did not affect this (Fig. 5). These results suggest that MRK-16 potentiates the MDR-reversing activity of SDZ PSC 833 by a different mechanism. We hypothesize that molecular interactions between Pgp and SDZ PSC 833 may be modulated by MRK-16. Such ternary molecular interactions are now under investigation.

The potentiation of the MDR-reversing effect of SDZ PSC 833 and CsA by MRK-16 was also observed in vivo in mice bearing drug-resistant HCT-15 human colon cancer cells. Since MRK-16 promotes an immune response to MDR tumor cells, immunological therapeutic effects could be involved in combination therapy. Therefore, the ternary combination of antitumor drugs, SDZ PSC 833, and MRK-16 may provide a more effective modality to overcome MDR in vivo.

Acknowledgements We are grateful to Dr. O. Yano (Mitsui Pharmaceuticals) and Dr. T. Watanabe (Sandoz Pharmaceuticals) for their helpful discussions on this work. This work was supported by a special grant for Advanced Research on Cancer, Grants-in-Aid for Cancer Research from the Ministry of Education, Science, and Culture, Japan, and also by the Uehara Memorial Foundation.

References

1. Archinal-Mattheis A, Rzepka RW, Watanabe T, Kokubu N, Itoh Y, Combates NJ, Bair KW, Cohen D (1995) Analysis of the interaction of SDZ PSC 833 ([3′-keto-Bmt1]-Val2]-cyclosporin), a multidrug resistance modulator, with P-glycoprotein. Oncol Res 7:603
2. Baba M, Nakanishi O, Sato W, Saito A, Miyama Y, Yano O, Shimada S, Fukazawa N, Naito M, Tsuruo T (1995) Relationship between multidrug resistant gene expression and multidrug resistant-reversing effect of MS-209 in various tumor cells. Cancer Chemother Pharmacol 36:361
3. Boesch D, Gaveriaux C, Jachez B, Pourtier-Manzanedo A, Bollinger P, Loor F (1991) In vivo circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. Cancer Res 51:4226
4. Boesch D, Muller K, Pourtier-Manzanedo A, Loor F (1991) Restoration of daunomycin retention in multidrug-resistant P388 cells by submicromolar concentrations of SDZ PSC 833, a non-immunosuppressive cyclosporin derivative. Exp Cell Res 196:26
5. Dalton WS, Grogan TM, Meltzer PS, Scheper RJ, Durie BGM, Taylor CW, Miller TP, Salmon SE (1989) Drug-resistance in multiple myeloma and non-Hodgkin's lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. J Clin Oncol 7:415
6. Endicott JA, Ling V (1989) The biochemistry of P-glycoprotein-mediated multidrug resistance. Annu Rev Biochem 58:137
7. Keller RP, Altermatt HJ, Nooter K, Poschman G, Laissue JA, Bollinger P, Hiestand PC (1992) SDZ PSC 833, a non-immunosuppressive cyclosporin: its potency in overcoming P-glycoprotein-mediated multidrug resistance of murine leukemia. Int J Cancer 50:593
8. Keller RP, Altermatt HJ, Donatsch P, Zihlmann H, Laissue JA, Hiestand PC (1992) Pharmacologic interactions between the resistance-modifying cyclosporin SDZ PSC 833 and etoposide (VP 16-213) enhance in vivo cytostatic activity and toxicity. Int J Cancer 51:433
9. Miller TP, Grogan TM, Dalton WS, Spier CM, Scheper RJ, Salmon SE (1991) P-glycoprotein expression in malignant lymphoma and reversal of clinical drug resistance with chemotherapy plus high-dose verapamil. J Clin Oncol 9:17
10. Naito M, Tsuge H, Kuroko C, Koyama T, Tomida A, Tatsuta T, Heike Y, Tsuruo T (1993) Enhancement of cellular accumulation of cyclosporin by anti-P-glycoprotein monoclonal antibody MRK-16 and synergistic modulation of multidrug resistance. J Natl Cancer Inst 85:311
11. Naito M, Watanabe T, Tsuge H, Koyama T, Oh-hara T, Tsuruo T (1996) Potentiation of the reversal activity of SDZ PSC833 on multidrug resistance by an anti-P-glycoprotein monoclonal antibody, MRK-16. Int J Cancer 67:435
12. Pastan I, Gottesman MM (1987) Multiple-drug resistance in human cancer. N Engl J Med 316:1388
13. Saeki T, Ueda K, Tanigawara Y, Hori R, Komano T (1993) Human P-glycoprotein transports cyclosporin A and FK-506. J Biol Chem 268:6077
14. Sato W, Fukazawa N, Nakanishi O, Baba M, Suzuki T, Yano O, Naito M, Tsuruo T (1995) Reversal of multidrug resistance by a novel quinoline derivative, MS-209. Cancer Chemother Pharmacol 35:271
15. Shirai A, Naito M, Tatsuta T, Dong J, Hanaoka K, Mikami K, Ohhara T, Tsuruo T (1994) Transport of cyclosporin A across the brain capillary endothelial cell monolayer by P-glycoprotein. Biochim Biophys Acta 1222:400
16. Slater LM, Sweet P, Stupecky M, Gupta S (1986) Cyclosporin A reverses vincristine and daunorubicin resistance in acute lymphatic leukemia in vitro. J Clin Invest 77:1405
17. Slater LM, Sweet P, Stupecky M, Wetzel MW, Gupta S (1986) Cyclosporin A corrects daunorubicin resistance in Ehrlich ascites carcinoma. Br J Cancer 54:235
18. Sonneveld P, Durie BGM, Lokhorst HM, Marie JP, Solbu G, Sucin S, Zittoun R, Lswenberg B, Nooter K (1992) Modulation of multidrug-resistant multiple myeloma by cyclosporin. Lancet 340:255
19. Tsuruo T (1988) Mechanism of multidrug resistance and implications for therapy. Jpn J Cancer Res 79:285
20. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y (1981) Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res 41:1967

21. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y (1982) Increased accumulation of vincristine and adriamycin in drug-resistant tumor cells following incubation with calcium antagonists and calmodulin inhibitors. Cancer Res 42:4730
22. Tsuruo T, Iida-Saito H, Kawabata H, Oh-hara T, Hamada H, Utakoji T (1986) Characteristics of resistance to Adriamycin in human myelogenous leukemia K562 resistant to Adriamycin and in isolated clones. Jpn J Cancer Res 77:682
23. Twentyman PR (1988) Modification of cytotoxic drug resistance by non-immuno-suppressive cyclosporins. Br J Cancer 57:254
24. Watanabe T, Tsuge H, Oh-hara T, Naito M, Tsuruo T (1995) Comparative study on reversal efficacy of SDZ PSC 833, cyclosporin A and verapamil on multidrug resistance in vitro and in vivo. Acta Oncol 34:235
25. Watanabe T, Naito M, Oh-hara T, Itoh Y, Cohen D, Tsuruo T (1996) Modulation of multidrug resistance by SDZ PSC 833 in leukemic and solid tumor-bearing mice model. Jpn J Cancer Res 87:184
26. Yusa K, Tsuruo T (1989) Reversal mechanism of multidrug resistance by verapamil: direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells. Cancer Res 49:5002