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Review paper

Multidrug resistance

Takashi Tsuruo and Akihiro Tomida

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Yayoi, Bunkyo, Tokyo 113, Japan.
Tel: (+81) 3 3812 2111 extn 7861; Fax: (+81) 3 3816 3592.

Since we found verapamil as a multidrug resistance (MDR) reversing agent in 1981, many MDR reversing compounds have been reported. This type of drug must have strong effects with little side effects. We recently found MS-209 and PSC-833 as reversing agents. These two compounds interacted directly with P-glycoprotein, and showed a good MDR reversing effect *in vitro* and *in vivo*. MRK16, an antibody against P-glycoprotein, also showed a good therapeutic effect against drug resistant human tumors. MS-209, PSC-833 and the antibody against P-glycoprotein are interesting candidates for clinical use in the future.

Key words: MDR reversing agent, monoclonal antibody, MS-209, PSC-833.

Introduction

Resistance of tumors to a variety of chemotherapeutic agents presents a major problem in cancer treatment. Resistance to such agents as adriamycin (ADM), Vinca alkaloids and actinomycin D can be acquired by tumor cells after treatment with a single drug.[1] The gene responsible for multidrug resistance (MDR), termed *mdr1*, encodes a particular type of membrane glycoprotein (P-glycoprotein) that pumps out various cytotoxic drugs from the cell.[2] The P-glycoprotein has been shown to bind anticancer drugs[3,4] and to be an ATPase[5,6] localized at the plasma membrane of resistant cells.[7] Transfection of cloned *mdr1* genes confers MDR on sensitive cells.[8–10]

The amount of P-glycoprotein expressed in cells has been measured in tumor samples, and was found to be elevated in intrinsically drug-resistant cancers of the colon, kidney and adrenal, as well as in some tumors that acquired drug resistance after chemotherapy.[11–15] The expression of the *mdr1* gene in tumors correlates well with clinical drug resistance.[16,17] In addition to tumor cells, P-glycoprotein is also expressed in various normal tissues such as adrenal, gravid uterus, kidney, liver, colon and capillary endothelium in brain.[18–24] P-glycoprotein expressed in such normal tissues could have physiologic functions specific to respective tissues.

Because P-glycoprotein appears to be involved in both acquired MDR and intrinsic drug resistance in human cancer, the selective killing of tumor cells expressing P-glycoprotein could be very important for cancer therapy, although the side effects on normal cells expressing P-glycoprotein must be considered carefully. In an effort to devise effective treatments for human drug-resistant cancers, several approaches have been developed to circumvent the MDR of tumor cells, including (i) application of chemosensitizing agents and (ii) application of monoclonal antibodies (mAbs) against P-glycoprotein. These therapeutic approaches are described in this review.

MDR reversing agents

In 1981, we reported that the calcium channel blocker verapamil inhibited active drug efflux and restored drug sensitivity in MDR cells.[25] Various compounds, including calcium channel blockers and calmodulin inhibitors, have been shown to enhance the cytotoxic activity of various antitumor agents (refs 25–31, 37; for review, see refs 32 and 33). Verapamil was reported to be a good inhibitor of the photoaffinity labeling of P-glycoprotein with a vinblastine photoanalog[3,4] and of the transport of Vinca alkaloid into vesicles from MDR cells.[34,35] Direct binding of verapamil to P-glycoprotein was shown using MDR Chinese hamster lung and human K562 cells.[36,37] Verapamil was actively effluxed from resistant cells.[37] These findings suggest that verapamil competitively inhibits the transport of antitumor agents by P-glycoprotein, resulting in the reversal of MDR.

Correspondence to T Tsuruo

Preliminary clinical studies have been carried out combining calcium channel blockers with chemotherapy. The combination chemotherapy was potentially useful against refractory acute lymphocytic leukemia of children, malignant lymphoma, multiple myeloma, various advanced solid tumors and small cell lung cancer, but induced side effects such as reversible hypotension and arrhythmias.

Cyclosporin A, an immunosuppressive drug, is another chemosensitizer that has been well studied[38,39] and used for clinical study to overcome drug resistance. Cyclosporin A is also a competitive inhibitor of drug transport by P-glycoprotein.[39] Clinical combination chemotherapy was carried out with cyclosporin A on refractory multiple myeloma patients.[40] Nine out of 21 patients responded to the combination chemotherapy with cyclosporin A. Interestingly, the *mdr1* gene was detected in 12 of 15 patients tested before the treatment and seven MDR-positive patients responded to the treatment while no MDR-negative patients did. The clinical studies with verapamil and cyclosporin A suggest that combination chemotherapy with chemosensitizers is potentially effective to overcome clinical drug resistance, especially in patients with resistant tumors expressing P-glycoprotein.

MS-073 is a quinoline derivative that is effective to overcome MDR *in vitro* and *in vivo*.[41] MS-073 at 0.1 μM almost completely reversed resistance to vincristine (VCR) in VCR-resistant P388 (P388/VCR) cells *in vitro*. The compound also reversed the resistance *in vitro* against VCR, ADM, etoposide and actinomycin D in ADM-resistant human myelogenous K562 (K562/ADM) cells, ADM-resistant human ovarian carcinoma A2780 cells and colchicine-resistant human KB cells. MS-073 administered i.p. daily for 5 days with VCR enhanced the chemotherapeutic effect of VCR in VCR-resistant P388-bearing mice. Increases in life span of 19–52% were obtained by the combination of 100 μg/kg VCR with 3–100 mg/kg MS-073, as compared with the control. The ability of MS-073 to reverse MDR was higher, especially at low MS-073 doses, than that of verapamil, both *in vitro* and *in vivo*. MS-073 enhanced accumulation of [^{3}H]VCR in K562/ADM cells. Photolabeling of P-glycoprotein with 200 nM [^{3}H]azidopine in K562/ADM plasma membranes was completely inhibited by 10 μM MS-073, indicating that MS-073 reverses MDR by competitively inhibiting drug binding to P-glycoprotein.

FK-506, a novel immunosuppressive agent, was found to be an interesting compound. FK-506 at 3 and 10 μg/ml completely reversed the resistance against VCR *in vitro* in VCR- and ADM-resistant P388 leukemia, and human ovarian cancer A2780 cells.[42] FK-506 also enhanced the cytotoxicity of VCR in highly resistant human K562/ADM *in vitro* and enhanced the chemotherapeutic effect of VCR in P388/VCR-bearing mice. When 20 mg/kg of the compound was combined with 200 mg/kg of VCR, 151% of the T/C value was obtained. Under the protocol used in this study, FK-506 was more potent than cyclosporin A and verapamil. FK-506 efficiently inhibited [^{3}H]azidopine binding to P-glycoprotein. The binding of VCR to K562/ADM plasma membrane was inhibited by FK-506 as effectively as cyclosporin A. Moreover, the accumulation of VCR in ADM-resistant human ovarian cells (2780AD) was increased by FK-506 as efficiently as cyclosporin A and verapamil.

PSC833 is a non-immunosuppressive analog of cyclosporin A that shows superior effects to cyclosporin A in overcoming MDR *in vitro* and *in vivo*.[43–45] It was shown to be 10-fold more effective than cyclosporin A *in vitro*. Moreover, PSC833 surpassed cyclosporin A and verapamil in the prolongation of the life span of P388/VCR-bearing mice when chemosensitizers were orally administered with i.p. or i.v. administration of VCR and ADM. PSC833 inhibited [^{3}H]azidopine binding to P-glycoprotein as well as cyclosporin A, and increased cellular accumulation of VCR and ADM in resistant tumors with a lower concentration than cyclosporin A.

MS-209—a quinoline derivative for the reversal of MDR

MS-209, an analog of MS-073, is considered for clinical application because of its efficient oral bioavailability and potentiating effects.[46] 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 ADM-resistant P388 (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 μM to the MDR P388 cells, MS-209 at 1 and 3 μM completely reversed VCR resistance in P388/VCR and P388/ADM cells, respectively. The sensitivity to VCR in the parental P388 cells was moderately enhanced by MS- 209. MS-209 also remarkably enhanced the sensitivity to VCR in the MDR K562 cells. K562/VCR and K562/ADM cells showed 171- and 1847-fold resistance, respectively, to VCR as compared with parental K562 cells. VCR resistance in MDR K562 cells was completely re-

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

MS-209	IC_{50} (ng/ml) of VCR[b]					
(μM)	P388	P388/VCR	P388/ADM	K562	K562/VCR	K562/ADM
0	1.89 (1)[c]	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	0.41 (4.6)	1.48 (81)	4.72 (16)	0.20 (1.7)	0.88 (66)	19.3 (33)
3	0.18 (11)	0.20 (273)	0.92 (82)	0.17 (2.0)	0.55 (106)	2.27 (277)
10	<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.
[b] Each value represents the mean of triplicate determinations. Standard deviations were within 10% of each value.
[c] Numbers in parentheses represent relative values as compared with the IC_{50} for each parent cell line in the absence of MS-209.

versed by 10 μM MS-209. A similar potentiating effect of MS-209 on ADM cytotoxicity was observed in the MDR P388 and K562 cells.

We examined the combined chemotherapeutic effect of ADM and MS-209 on P388/ADM-bearing mice. As shown in Table 2, oral administration of MS-209 with ADM given i.p. once a day for five consecutive days starting from day 1 apparently increased the life span of P388/ADM-bearing mice. The most prominent results were obtained when MS-209 was administered with 2 mg/kg ADM. T/C values of 111–144 and 150–194% were obtained with combined treatment with 50–200 mg/kg MS-209 (experiment 1) and 200–450 mg/kg MS-209 (experiment 2), respectively.

As shown in Figure 1, [^{3}H]azidopine specifically labeled an M_r 170 000–180 000 protein in K562/ADM cells but not in drug-sensitive K562 cells. In the presence of 100 μM 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.

Together with other pharmacological and toxicological profiles (data not shown), the present results

Table 2. Effect of MS-209 on 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 (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
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] $CD2F_1$ mice were given i.p. implants of 10^6 P388/VCR leukemia cells on day 0. MS-209 was administered p.o. once a day just before i.p. injection of ADM from days 1 to 5.
[b] T/C value: median survival time of treated mice divided by median survival time of control mice.
[c] Difference in body weight (g) between days 5 and 1.

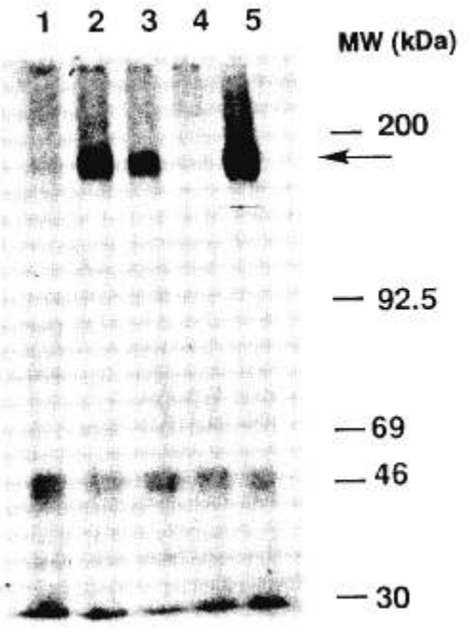

Figure 1. Inhibition of [^{3}H]azidopine photolabeling of P-glycoprotein. K562 (lane 1) and K562/ADM (lanes 2–5) membrane vesicles (50 μg of protein) were incubated with 200 nM [^{3}H]azidopine in the absence (lanes 1 and 5) or presence of MS-209 at 1 (lane 2), 10 (lane 3) or 100 μM (lane 4). After solubilization,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

suggest that MS-209 will be an orally-active MDR reversing drug.

mAbs

During the past 5 years, our group was developed immunotherapeutic approaches for overcoming MDR cancers using anti-P-glycoprotein mAbs, MRK16 and MRK17.[47] We have evaluated the effect of MRK16 and MRK17 on the growth of human MDR ovarian cancer cells 2780AD.[48] 2780AD cells (10^7/mouse) were inoculated subcutaneously in athymic mice. In the experiment, we demonstrated growth inhibition of the mAbs. Animals received intravenous injection of various amounts of MRK16 or MRK17 on days 2 and 7 after tumor inoculation. Subcutaneous tumors never developed in the mice treated with 10 μg or more per animal of MRK16. When the mice were treated with 1 μg per animal of MRK16 or 1 or 10 μg per animal of MRK17, the development of tumors was substantially retarded as compared with control mice where palpable tumors developed by day 20.

In the next experiment, the therapy with antibodies was started after the tumors became palpable (150–400 mm^3 in volume). One group of animals received 6 mg/kg of adriamycin intravenously three times as control therapy. Animals received various amounts of MRK16 or MRK17 intravenously on days 20, 23 and 26, and the average tumor volume was measured. At either 1 or 2 days after the first injection of MRK16, the size of the tumor masses remarkably decreased in all five mice. After the third MRK16 injection, the average tumor mass was substantially reduced and in four out of five mice the tumor mass had completely disappeared. Among these four mice, a palpable tumor recurred in one mouse on day 42; the other three mice remained tumor-free up to day 60. No significant inhibition of tumor growth was observed in animals which received 1 mg MRK17 intravenously on days 20, 23 and 26. These mAbs had no effect on the *in vivo* growth of drug-sensitive parent tumor A2780 cells, suggesting that the effect of these antibodies was specific for the drug-resistant tumor cells over-expressing the P-glycoprotein.

Several mechanisms, such as direct cytotoxicities of the mAbs or immune response induced by mAbs, may be involved in the growth inhibition of the xenografts. Complement-dependent cytotoxicity for 2780AD cells was clearly demonstrated *in vitro* using MRK16 and rabbit serum as complement. Antibody-dependent cell-mediated cytolysis by MRK16 was more clearly demonstrated than by MRK17. The benefit of MRK16 use *in vivo* in MDR cancer treatment has also been verified in the model of xenograft of MDR human colon cancer (HT29mdr1).[49]

Conclusion

In this review, we have described therapeutic approaches to overcome MDR in cancer cells. Several new antitumor drugs were demonstrated to be effective for the treatment of resistant tumors *in vitro* and *in vivo*. As P-glycoprotein plays a central role in MDR, the therapeutic approaches targeting P-glycoprotein, such as application of chemosensitizing drugs and mAbs against P-glycoprotein, have been studied. Chemosensitizing drugs such as verapamil and cyclosporin A competitively inhibit the efflux of antitumor drugs from the cells by P-glycoprotein. Combination chemotherapy with antitumor agents and chemosensitizers could actually overcome clinical drug resistance in some refractory cancer patients. Several new chemosensitizing drugs have

been developed and some of them are under clinical phase study in Japan, the US and Europe. mAbs against P-glycoprotein are also attractive tools for overcoming MDR mediated by P-glycoprotein. Some antibodies, such as MRK-16, enhanced the immune responses to resistant tumors possessing P-glycoprotein. They also modulated the transport function of P-glycoprotein, and could be combined with antitumor drugs and chemosensitizers.

In the trials to reverse MDR mediated by P-glycoprotein, we must be concerned about the physiologic functions of P-glycoprotein expressed in various normal tissues. P-glycoprotein expressed in brain capillary endothelium could be functionally involved in the blood–brain barrier.[50–52] P-glycoprotein in adrenal gland could be responsible for secretion of steroid hormones,[53–54] P-glycoprotein in the luminal surface of the colon, brush border of proximal tubules in kidney and biliary canalicular surface of hepatocytes could contribute to the excretion of natural toxic substances into the lumen of the gastrointestinal tract, urine and bile, respectively.[55]

Although the modulation of MDR is not easy to achieve, the therapeutic approaches described here are interesting. Further studies are needed to develop effective modalities to overcome drug resistance.

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