RAPID COMMUNICATION Jpn. J. Cancer Res. 84, 489–492, May 1993

Enhancement of Reversing Effect of Cyclosporin A on Vincristine Resistance by Anti-P-glycoprotein Monoclonal Antibody MRK-16

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The synergistic effect of MRK-16, a monoclonal antibody against P-glycoprotein, and cyclosporin A (CsA) on the modulation of vincristine resistance was studied by isobologram analysis in three different, highly multidrug-resistant tumor cells. In all cell lines, the synergistic effect was demonstrated at various concentrations of MRK-16 and CsA. While MRK-16 alone did not enhance the sensitivity of the moderately resistant KB-8-5 cells to vincristine, it increased two-fold the reversing effect of cyclosporin A at 1 μM, an achievable blood concentration. Since MRK-16 alone showed therapeutic effects against multidrug-resistant tumors, the combined use of MRK-16, CsA and antitumor agents should provide therapeutic benefits for the treatment of resistant tumors.

Key words: VCR resistance — MRK-16 — Cyclosporin A

Drug resistance is a major obstacle to successful cancer chemotherapy. Tumors initially sensitive to chemotherapy often recur and show cross-resistance to a variety of antitumor agents. The mechanism of multidrug resistance has been well studied, and it was found that P-glycoprotein, an efflux pump for hydrophobic antitumor agents, plays a key role in rendering tumor cells resistant to various antitumor agents.[1-3] A number of compounds, including cyclosporin A (CsA)[4,5] have been reported to reverse multidrug resistance when combined with antitumor agents. CsA competitively inhibits the binding of antitumor agents to P-glycoprotein, increases the intracellular accumulation of antitumor agents in the resistant cells, and overcomes drug resistance.[6,7] Recently, it was reported that CsA, to some extent, circumvented clinical drug resistance to the VAD (vincristine, doxorubicin and dexamethasone) regimen in multiple myeloma.[8]

We recently reported that MRK-16, a monoclonal antibody that recognizes the extracellular domain of human P-glycoprotein,[9] increased cellular accumulation of CsA, and enhanced the reversing effect of CsA in adriamycin (ADM)-resistant human myelogenous leukemia K562 (K562/ADM) cells.[10] In this study, we examined the synergistic effect of MRK-16 and CsA on the modulation of vincristine (VCR) resistance with two different highly multidrug-resistant solid tumors and a moderately resistant solid tumor cell line.

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All experiments were carried out in triplicate and repeated at least twice. In KB-C4 cells,[11] for example, growth-inhibitory effects of MRK-16 and CsA alone and in combination were examined in the presence of 300 nM VCR. The 300 nM VCR inhibited the growth of KB-C4 cells by 44%. We analyzed the combined effects of MRK-16 and CsA at the IC_{50}. Based on the individual dose-response curves of MRK-16 and CsA, three isoeffect curves (mode I, solid line; mode II (MRK-16), chain line; mode II (CsA), dotted line) were drawn as follows.[12-15] Mode I line: When a dose of MRK-16 is chosen, there remains an increment in isoeffect to be produced by CsA. If the two agents act independently, the addition is performed by taking the increment in doses, starting at zero, that gives survivals that add up to IC_{50}. Mode II (MRK-16) line: When the dose of MRK-16 is chosen, an isoeffect curve can also be calculated by taking the dose increment of CsA that gives the required contribution to the total effect up to IC_{50}. Mode II (CsA) line: Similarly, when the dose of CsA is chosen, an isoeffect curve can be calculated by taking the dose increment of MRK-16 that gives the required contribution to IC_{50}. The experimental data points of the combination of MRK-16 and CsA were plotted. When the data point is within the area enclosed by the three isoeffect curves (envelope of additivity), the combination is considered to be additive. When the data point falls to the left of the envelope, the combination of MRK-16 and CsA can be considered synergistic.

Fig. 1a shows the effect of MRK-16 on the growth-inhibitory activity of VCR at 300 nM against KB-C4

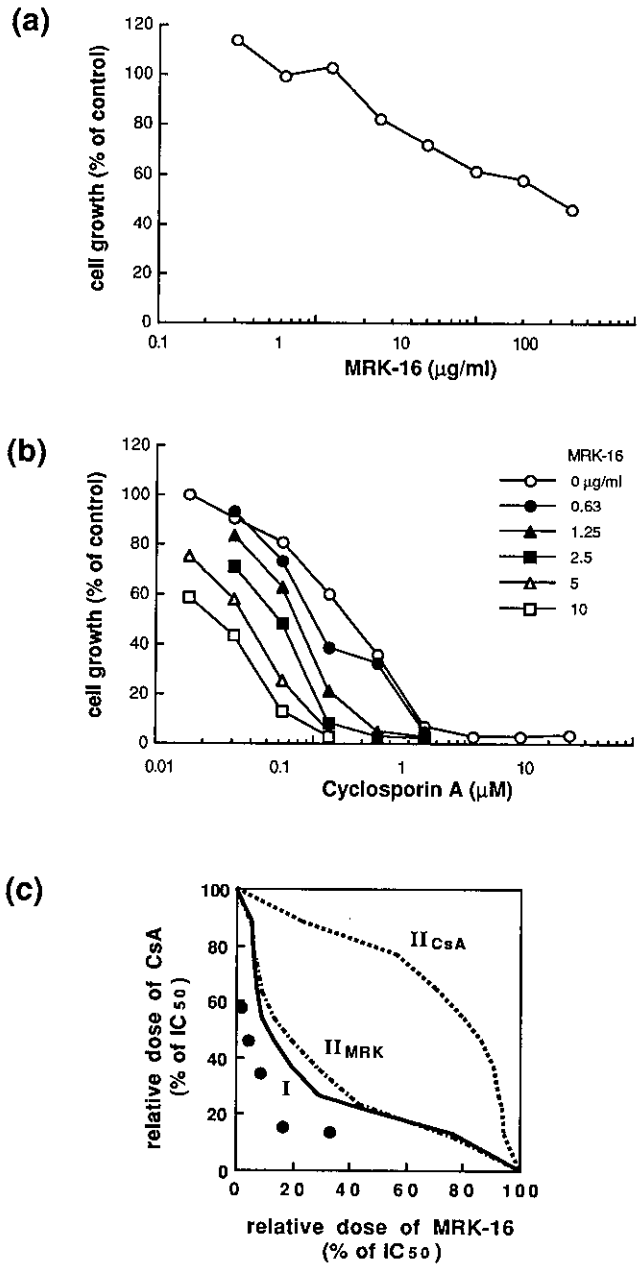

Fig. 1. Isobologram analysis of the synergism between MRK-16 and CsA in the sensitization of KB-C4 cells to VCR. (a) Growth-inhibitory effect of MRK-16 in KB-C4 cells in the presence of 300 n*M* VCR. (b) Growth-inhibitory effect of CsA in KB-C4 cells in combination with MRK-16 (at the indicated concentrations) in the presence of 300 n*M* VCR. Data are means of triplicate determinations. SDs were less than 5% of mean values. (c) Three isoeffect curves (mode I, solid line; mode II (MRK-16), chain line; mode II (CsA), dotted line) were drawn according to the individual dose-response curves of MRK-16 and CsA presented in a and b, respectively, and the experimental data points of the combination of MRK-16 and CsA were plotted.

cells. The 50% reduction of cell growth was attained at 30 μg/ml of MRK-16. Fig. 1b shows the effect of CsA, with or without various concentrations of MRK-16, on the growth-inhibitory activity of 300 n*M* VCR. The concentration of CsA needed for 50% inhibition of cell growth was reduced when CsA and MRK-16 were combined. Based on the individual dose-response curves of MRK-16 and CsA shown in Fig. 1a and 1b, respectively, three isoeffect curves of 50% growth inhibition (mode I. solid line; mode II (MRK-16), chain line; mode II (CsA), dotted line) were drawn and the experimental data points of the combination of MRK-16 and CsA were plotted (Fig. 1c). The data points fell on the left side of the envelope of additivity, indicating the synergistic interaction of MRK-16 and CsA in the enhancement of VCR sensitivity in KB-C4 cells. Similar isobologram analyses were carried out using other multidrug-resistant tumor cells 2780^{AD} [16] and K562/ADM.[17] As shown in Fig. 2, the data points fell on the left side of the envelope in both experiments. These results indicate that MRK-16 and CsA synergistically modulate VCR resistance in various multidrug-resistant tumor cells that express P-glycoprotein.

In the clinical context, even moderate resistance is a serious problem in cancer chemotherapy. Therefore, the combined effect of MRK-16 and CsA was further examined in the moderately multidrug-resistant cell line KB-8-5,[11] which shows 31-fold increased resistance to VCR, as compared to the parental KB-3-1 cells (Table I). In this cell line, the isobologram analysis could not be carried out because MRK-16 was unable to modulate VCR resistance. Therefore IC_{50} values of VCR in the presence of graded concentrations of CsA were compared to those treated with the CsA/MRK-16 combination. While CsA at 0.3, 1, and 3 μM enhanced the cytotoxicity of VCR 3.1-, 11-, and 31-fold, respectively, the combinaion of 10 μg/ml MRK-16 with 0.3, 1, and 3 μM CsA enhanced the cytotoxicity 4.6-, 23- and 33-fold, respectively. According to the effect multiplication criterion,[18] the values at 0.3 and 1 μM CsA were greater than expected. Thus the synergism of MRK-16 and CsA was also demonstrated in the moderately multidrug-resistant KB-8-5 cells. Since 1 μg/ml of CsA (0.83 μM) in blood is a therapeutically achievable level for immunosuppression without excessive dose-related toxicity,[19,20] and, actually, the clinical drug resistance was circumvented at this concentration of CsA,[8] the enhancement of the reversing effect of CsA at 0.3 and 1 μM by MRK-16 has an interesting clinical implication.

We previously reported that MRK-16 promotes monocyte-, lymphocyte-, and complement-dependent multidrug-resistant tumor cell killing *in vitro*,[21] and intravenous administration of MRK-16 induces the regression of resistant tumors that developed subcutaneously in

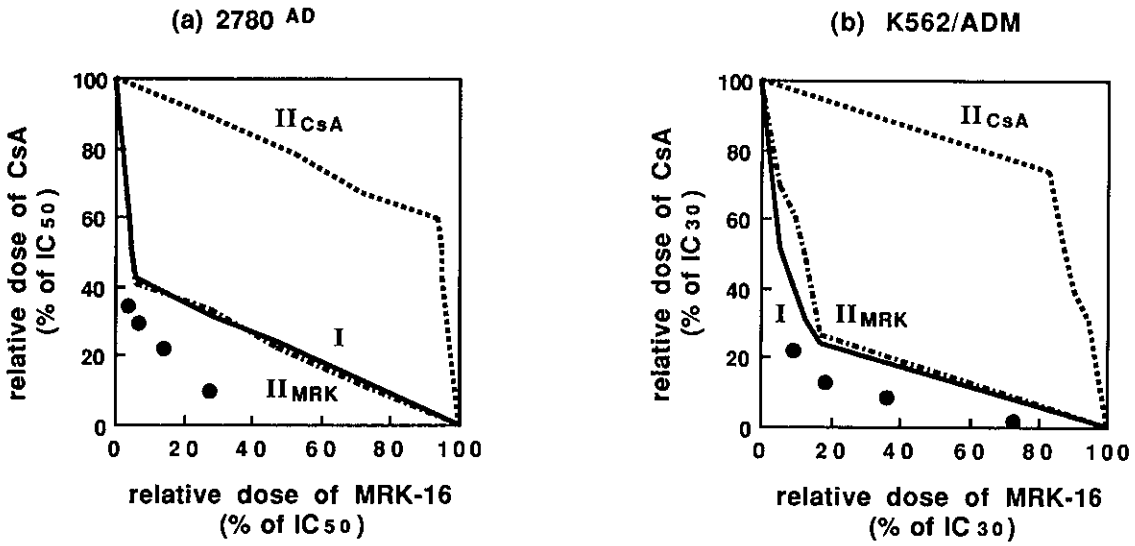

Fig. 2. Isobologram of MRK-16 in combination with CsA in the modulation of VCR resistance. The combined effect of MRK-16 with CsA in the modulation of VCR resistance in 2780^{AD} (a) and K562/ADM (b) cells was analyzed by constructing an envelope of additivity at the point of IC_{50} (2780^{AD}) or IC_{30} (K562/ADM). Since MRK-16 at up to 80 μg/ml did not inhibit the growth of K562/ADM cells by 50%, the combined effect of MRK-16 and CsA was analyzed at the point of IC_{30} in this cell line. IC_{50} values of MRK-16 and CsA in the presence of 300 nM VCR were 18.0 μg/ml and 0.54 μM in 2780^{AD} cells, respectively. IC_{30} of MRK-16 and CsA in the presence of 300 nM VCR were 10.6 μg/ml and 1.05 μM in K562/ADM cells, respectively.

Table I. Synergistic Reversal of VCR Resistance in KB-8-5 Cells by Treatment with Combined MRK-16 and CsA

Cell line	CsA (μM)	IC_{50} of VCR (nM)	
		MRK-16 (−)	MRK-16 (+)
KB-3-1	0	1.8 (1)[a]	1.8 (1)
	0.3	1.6 (1.1)	1.7 (1.1)
	1	1.6 (1.1)	1.7 (1.1)
KB-8-5	3	1.4 (1.3)	1.5 (1.2)
	0	56 (1)	55 (1)
	0.3	18 (3.1)	12 (4.6)[b]
	1	4.9 (11)	2.4 (23)[b]
	3	1.8 (31)	1.7 (33)

KB-3-1 and KB-8-5 cells were treated with graded concentrations of VCR in the presence or absence of the indicated concentrations of CsA combined with or without 10 μg/ml of MRK-16; the IC_{50} value of VCR was then determined. SDs were less than 5% of mean values.

a) Numbers in parentheses indicate the fold decrease in the IC_{50} value as compared to that obtained in the absence of MRK-16 and CsA.

b) Synergistic as determined by the effect multiplication method.[18]

athymic mice.[22] Although CsA at 1 μg/ml efficiently prevents development of cytotoxic T lymphocytes, it does not suppress the killing activity of lymphocytes and macrophages against tumor cells *in vitro* (S. Yano *et al.*, unpublished observations). We assume that combined use of MRK-16 and CsA might have a dual advantage, namely the enhancement of antitumor immune responses and the synergistic enhancement of antitumor agent cytotoxicity, in the treatment of resistant tumors expressing P-glycoprotein.

This work was supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan. We are grateful to Dr. I. Pastan (National Cancer Institute, NIH) for providing multidrug-resistant KB carcinoma cells (KB-8-5 and KB-C4) and to Dr. R. Ozols (National Cancer Institute, NIH) for multidrug-resistant ovatian cancer cell line (2780^{AD}). We are indebted to Sandoz Pharmaceuticals Co., Ltd. for CsA and to Eli Lilly Japan Co., Ltd. for VCR. We also thank E. Tsuruo and S. Yachida for assistance in the preparation of the manuscript.

(Received January 13, 1993/Accepted March 2, 1993)

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