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Reversal of multidrug resistance by an immunosuppressive agent FK-506*

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Summary. FK-506, a novel immunosuppressive agent, was examined for its reversing effect on multidrug-resistant tumor cells. FK-506 at 3 μM completely reversed the resistance against vincristine (VCR) in vitro in VCR-resistant mouse leukemia P388 cells (P388/VCR). FK-506 also enhanced the cytotoxicity of VCR in Adriamycin(ADM)-resistant human ovarian cancer A2780 cells (AD_{10}) and ADM-resistant human myelogenous leukemia K562 cells (K562/ADM) in vitro. FK-506 was also effective in modulating sensitivity to ADM in AD_{10} cells in vitro. FK-506 enhanced the chemotherapeutic effect of VCR in P388/VCR-bearing mice. When 20 mg/kg FK-506 was combined with 200 μg/kg VCR, a T/C value of 151% was obtained. Under the protocol used in this study, FK-506 was more potent than cyclosporin A (CsA) and verapamil. FK-506 inhibited [^{3}H]azidopine binding to P-glycoprotein efficiently. The binding of VCR to K562/ADM plasma membrane was inhibited by FK-506 as effectively as by CsA. Moreover, the accumulation of VCR in AD_{10} cells was increased by FK-506 as efficiently as that of CsA and verapamil. These results indicate that FK-506 directly interacts with P-glycoprotein like CsA and verapamil, inhibits the active efflux of vincristine from resistant cells, increases the vincristine accumulation in resistant cells, and thus overcomes multidrug resistance in vitro and in vivo.

Introduction

Cancer chemotherapy is often hampered by drug resistance during treatment. When tumor cells acquire resistance to naturally occurring antitumor agents such as *Vinca* alkaloids and anthracycline antibiotics, they may show cross-resistance to a variety of antitumor agents of natural origin. The mechanism of the multidrug resistance has been studied well, and it was found that a glycoprotein termed P-glycoprotein, an efflux pump for hydrophobic antitumor agents, is an important component in rendering tumor cells resistant to various antitumor agents (reviewed in [3, 10, 23]).

It is well known that verapamil can inhibit the efflux of antitumor agents from resistant cells and can overcome this form of drug resistance [18, 19, 20]. Since the discovery of verapamil, many other compounds including cyclosporin A (CsA) were also reported to overcome multidrug resistance when combined with antitumor agents [14, 25] (see [24] for review). CsA is an immunosuppressive agent used against rejection of transplanted tissues in recipients. It was reported that CsA suppressed the induction of cytotoxic T lymphocytes by suppressing the production of interleukin-2, interleukin-3 and interferon γ, and the expression of interleukin-2 receptors [1, 2, 11]. CsA is hydrophobic compound and has an affinity to P-glycoprotein [8]. CsA increases the accumulation of antitumor agents in resistant cells and circumvents drug resistance [13].

FK-506 is a newly developed immunosuppressive agent isolated from *Streptomyces tsukubaensis* [4–6, 16]. FK-506 is more potent than CsA in suppressing the production of interleukin-2, interleukin-3 and interferon γ, the expression of interleukin-2 receptors, and the rejection of transplants [4–6]. Both CsA and FK-506 bind and inhibit rotamases in various cells [15, 17]. FK-506 is a hydrophobic compound and has similar biological activity to CsA, although these two compounds have no structural similarity. We were interested in the effect of FK-506 on multidrug-resistant cells, and we found FK-506 is more effective than CsA in overcoming multidrug resistance in vivo.

Abbreviations: VCR, vincristine; ADM, Adriamycin; CsA, cyclosporin A; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl blue); T/C, mean survival time of treated group of mice divided by mean survival time of control group; T/V, mean survival time of treated group of mice divided by mean survival time of the group of mice treated with vincristine alone

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Table 1. Effect of FK-506 upon growth-inhibitory actions of vincristine (VCR) on multidrug-resistant cells[a]

Cells	Modifiers	IC_{50} of VCR (nM)[b] with a modifier concentration of					
		0	0.1 μM	0.3 μM	1 μM	3 μM	10 μM
A2780	None	1.8 (1)[c]					
	FK-506				2.2 (0.8)	1.8 (1)	1.0 (1.8)
	CsA[d]				1.6 (1.1)	1.1 (1.6)	0.8 (2.3)
	Verapamil				1.5 (1.2)	0.9 (2)	0.7 (2.6)
AD_{10}	None	2070 (1)					
	FK-506				1260 (1.6)	130 (16)	20 (104)
	CsA				470 (4.4)	25 (83)	5.2 (398)
	Verapamil				2200 (0.9)	450 (4.6)	410 (5)
K562	None	0.8 (1)					
	FK-506				0.7 (1.1)	0.8 (1)	0.3 (2.7)
	CsA				0.4 (2)	0.2 (4)	0.2 (4)
	Verapamil				0.5 (1.6)	0.4 (2)	0.2 (4)
K562/ADM	None	680 (1)					
	FK-506				510 (1.3)	38 (18)	2.5 (272)
	CsA				570 (1.2)	84 (8)	15 (45)
	Verapamil				44 (15)	5.9 (115)	8.9 (76)
P388	None	0.5 (1)					
	FK-506			0.6 (0.8)	0.5 (1)	0.4 (1.3)	
	CsA			0.3 (1.7)	0.2 (2.5)	0.2 (2.5)	
	Verapamil			0.4 (1.3)	0.3 (1.7)	0.2 (2.5)	
P388/VCR	None	20 (1)					
	FK-506			8.1 (2.5)	0.8 (25)	0.1 (200)	
	CsA		1.7 (12)	0.3 (67)			
	Verapamil			2.3 (8.7)	0.4 (50)	0.1 (200)	

[a] Cells were cultured for 24 h (A2780 and AD_{10}) or 5 h (K562, K562/ADM, P388 and P388/VCR) and then treated with graded concentrations of VCR in the absence or presence of the indicated modifier concentrations and reincubated for 72 h. Growth-inhibitory effects were evaluated by MTT (thiazolyl blue) assay as described in Materials and methods

[b] Each value represents the mean of triplicate determinations. Standard deviation was within 5% of each mean value

[c] Numbers in parentheses indicate the *x*-fold decrease in the IC_{50} value as compared with that obtained in the absence of modifiers

[d] CsA, cyclosporin A

Materials and methods

Drugs. The sources of materials used in this work were as follows: FK-506 from Fujisawa Pharmaceutical Co. Ltd., Osaka; CsA from Sandoz Pharmaceuticals Co. Ltd., Tokyo; verapamil from Eizai Co. Ltd., Tokyo; Adriamycin (ADM) from Kyowa Hakko Co. Ltd., Tokyo; vincristine (VCR) from Shionogi Co. Ltd., Osaka; [^{3}H]VCR (263 GBg/mmol) and [^{3}H]azidopine (1.67 TBg/mmol) from Amersham Japan Ltd., Tokyo; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl blue) (MTT) and ATP from Sigma Chemical Co. Ltd., St. Louis.

Cell culture and drug treatment. P388 leukemia cells were supplied by Simonsen Laboratories Inc. (Gilroy, Calif.), under the auspices of the National Cancer Institute (NIH, Bethesda, Md.). P388 cells resistant to VCR (P388/VCR) were kindly supplied by the Mammalian Genetics and Animal Products Section, NCI, NIH. The human myelogenous leukemia K562 cell line was provided by Dr. Ezaki; sublines resistant to ADM (K562/ADM) were established in our laboratory [21, 22]. The human ovarian cancer line A2780 and its ADM-resistant subline (AD_{10}) were provided by Drs. R. Ozols and T. Hamilton, Medicine Branch, NCI, NIH [12].

For the drug treatment experiments, tumor cells (10^3 cells for P388 and P388/VCR cells, 2×10^3 cells for A2780, AD_{10} and K562 cells and 3×10^3 cells for K562/ADM cells) were cultured at 37° C for 24 h in 96-well flat-bottom plates (for A2780 and AD_{10}, which grow on the surface of the plate) or for 5 h (for other cell lines, which grow in suspension) containing 100 μl growth medium (RPMI-1640 medium containing 5% fetal bovine serum and 100 μg/ml kanamycin) in a humidified atmosphere comprising 5% CO_2/95% air. The cells were then treated with a graded concentration of VCR (0.03–3000 nM) or ADM (1–3000 nM) and reincubated for 72 h in the presence of the drugs. After the incubation, 50 μl 1 mg/ml MTT was added to each well and further incubated for 4 h. Plates were centrifuged for 15 min and then the supernatant was aspirated. Dimethylsulfoxide (150 μl/well) was added to each well and the mixture was pipetted well to solubilize the formazan crystals. The plates were then read immediately at 540 nm [9]. Three samples were used for each drug concentration. In the control cultures, tumor cells grew exponentially during the incubation period. When the effects of modifiers (FK-506, CsA and verapamil) were examined, the indicated concentrations of modifiers were added together with VCR and the cells were incubated in the presence of antitumor agents and modifiers as above. The median concentration of drug necessary to inhibit the growth of tumor cells by 50% (IC_{50}) was determined by plotting the logarithm of the drug concentration versus the growth rate (percentage of control) of the treated cells. FK-506 alone at a higher concentration slightly inhibited the growth of tumor cells; 15%, 15%, 17% and 11% inhibition was observed at 10 μM FK-506 in A2780, AD_{10}, K562 and K562/ADM cells respectively, and 17% and 24% inhibition at 3 μM FK-506 in P388 and P388/VCR cells respectively.

Evaluation of antitumor activity. For evaluation of antitumor activity, 0.1 ml diluted ascites fluid containing 10^6 P388/VCR cells was transplanted i.p. into CD_2F_1 mice [18, 20]. Drugs were injected i.p. daily on days 1–5 after tumor inoculation. Five mice were used for each experimental group. Antitumor activity was evaluated by the mean survival of a group of mice and was also expressed by the mean survival time of the

Table 2. Effect of FK-506 upon growth-inhibitory actions of Adriamycin (ADM) on AD_{10} cells[a]

Cells	Modifiers	IC_{50} of ADM (nM)[b] with a modifier concentration of			
		0 μM	1 μM	3 μM	10 μM
A2780	None	10 (1)			
	FK-506		10 (1)	8.7 (1.1)	7.9 (1.3)
	CsA		8.7 (1.1)	7.5 (1.3)	8.8 (1.1)
	Verapamil		7.3 (1.4)	6.4 (1.6)	5.6 (1.8)
AD_{10}	None	1460 (1)			
	FK-506		2050 (0.7)	780 (1.9)	140 (11)
	CsA		390 (4)	120 (12)	36 (41)
	Verapamil		280 (5)	160 (9)	48 (30)

[a] Cells were cultured for 24 h and they were treated with graded concentrations of ADM in the absence or presence of indicated modifier concentrations. After incubation for 72 h, growth-inhibitory effects were evaluated by MTT assay as described in Materials and methods

[b] Each value represents the mean of triplicate determinations. Standard deviation was within 5% of each mean value

[c] Numbers in parentheses indicate the *x*-fold decrease in the IC_{50} value as compared with that obtained in the absence of modifiers

treated group of mice divided by the mean survival time of the control group (T/C) and the mean survival time of the treated group of mice divided by mean survival time of the group of mice treated with vincristine alone (T/V).

Intracellular accumulation of [^{3}H]VCR. A2780 and AD_{10} cells (10^6 cells) in growth medium were plated in Corning six-well tissue-culture clusters and incubated for 24 h at 37° C. The medium in each well was aspirated and 0.5 ml fresh growth medium containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid was added. Then [^{3}H]VCR (7.5 pmol) and the indicated concentrations of drugs were added. After incubation at 37° C for 2 h, the intracellular VCR accumulation was determined as described previously [19].

Binding inhibition assay of [^{3}H]VCR. Inhibition of binding of [^{3}H]VCR to a membrane preparation was measured by filtration methods as described previously [7, 8]. Plasma membrane prepared from K562/ADM cells (50 μg protein) was incubated at 25° C with 0.2 μM [^{3}H]VCR and 3 mM ATP in 10 mM TRIS/HCl (pH 7.4), 250 mM sucrose, and 5 mM $MgCl_2$ in a total volume of 50 μl. After 10 min, the reaction was stopped by the addition of 4 ml ice-cold buffer. Samples were collected by filtration on membrane filter (Millipore MF membrane; pore size, 0.22 μm) pretreated with 3% bovine serum albumin solution and were then washed with another 4 ml ice-cold buffer. By this method, about 60% of membrane proteins were recovered on the filter. The filters were dried and radioactivity on each filter was measured. Then the concentrations of agents needed for 50% inhibition (IC_{50}) were determined.

Photolabeling of plasma membrane. Plasma membrane from K562/ADM cells (50 μg protein) was preincubated with 177 nM [^{3}H]azidopine and 10 μM or 100 μM drugs for 15 min at 25° C in 50 μl 10 mM TRIS/HCl (pH 7.2), 150 mM NaCl buffer. The reaction mixture was then irradiated for 10 min with a 400-W mercury 365-nm lamp (model H400PL; Chiyoda Kohan Co. Ltd., Tokyo) at a distance of 10 cm at 0° C. Photolabeled membrane was then subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis using 4% – 20% gradient gels (Daiichi Chemicals Co. Ltd., Tokyo). The gel was fixed in 25% isopropyl alcohol/10% acetic acid, treated with the fluorographic reagent Amplfy (Amersham Japan Ltd.) for 30 min, dried, and then exposed to X-ray film for 1 week at –70° C as described previously [26].

Results

Enhanced cytotoxicity of VCR and ADM in multidrug-resistant cells by FK-506

We examined the sensitizing effect of FK-506 in multidrug-resistant cells and their parental cells against VCR (Table 1). AD_{10} cells showed 1150-fold resistance against VCR as compared to the parental A2780 cells, and the IC_{50} values of VCR for A2780 and AD_{10} cells were 1.8 nM and 2070 nM respectively. When FK-506 was added at a final concentration of 1, 3 and 10 μM, the IC_{50} values of VCR in AD_{10} cells were shifted to 1260, 130 and 20 nM respectively. CsA enhanced the sensitivity to VCR in AD_{10} cells more effectively than FK-506, and verapamil was less effective than FK-506. The sensitivity to VCR in the parental A2780 cells was marginally modulated by FK-506; only 1.8-fold sensitization was observed at 10 μM FK-506. In K562/ADM cells, FK-506 at 3 μM and 10 μM shifted the IC_{50} values from 680 nM to 38 nM and 2.5 nM, respectively. Again FK-506 only marginally enhanced the sensitivity to VCR in the parental K562 cells. In the case of P388/VCR cells, FK-506 at 0.3, 1 and 3 μM shifted the IC_{50} values from 20 to 8.1, 0.8 and 0.1 nM, respectively. In this cell line CsA was more effective than FK-506, and the IC_{50} values were shifted to 1.7 nM and 0.3 nM by 0.1 μM and 0.3 μM CsA respectively. In the parental P388 cells, FK-506 was not effective in modulating the sensitivity to VCR. These results indicate that FK-506 as well as CsA and verapamil specifically enhanced the sensitivity to VCR in multidrug-resistant tumor cells.

FK-506 also enhanced the sensitivity to ADM in AD_{10} cells (Table 2). FK-506 at 3 μM and 10 μM shifted the IC_{50} values of ADM in AD_{10} cells from 1460 μM to 780 μM and 140 nM, respectively. The enhancement of the sensitivity to ADM was specific to the resistant cells and the IC_{50} value of ADM in the parental A2780 cells was not shifted by FK-506. Thus, FK-506 can modulate the multidrug resistance as well as CsA and verapamil in vitro.

Combined chemotherapeutic effect of VCR and FK-506 on P388/VCR-bearing mice

VCR administered daily for 5 days starting from day 1 showed a slight chemotherapeutic effect against P388/VCR-bearing mice (Table 3). T/C values were 109%, 109% and 111% at a VCR dosage of 100 μg/kg, 150 μg/kg and 200 μg/kg, respectively. FK-506 alone and

Table 3. Effect of FK-506 on antitumor activity of VCR in P388/VCR-bearing mice[a]

Drug and dosage	Survival time (days)	T/C (%)	T/V (%)[b]
Control	11.0±0[c]	100	
FK-506 (20 mg/kg)	12.5±0.6	114*[1]	
(10 mg/kg)	12.5±0.6	114*[1]	
(5 mg/kg)	12.8±0.5	116*[1]	
Cyclosporin A (20 mg/kg)	12.8±0.5	116*[1]	
(10 mg/kg)	12.3±0.5	112*[1]	
(5 mg/kg)	11.3±0.5	103*[1]	
Verapamil (75 mg/kg)	10.3±0.5	94*[2]	
(50 mg/kg)	10.5±0.6	96	
VCR (200 μg/kg)	12.2±0.4	111*[1]	100
+ FK-506 (20 mg/kg)	16.6±1.1	151*[1]	138*[3]
(10 mg/kg)	16.2±0.8	147*[1]	135*[3]
(5 mg/kg)	15.4±1.1	140*[1]	126*[3]
+ Cyclosporin A (20 mg/kg)	8.0±1.0	73*[1]	66*[3]
(10 mg/kg)	8.0±0.7	73*[1]	66*[3]
(5 mg/kg)	12.2±2.6	111	100
+ Verapamil (75 mg/kg)	13.6±0.9	124*[1]	112*[5]
(50 mg/kg)	13.4±0.9	122*[1]	110*[5]
VCR (150 μg/kg)	12.0±0	109*[1]	100
+ FK506 (20 mg/kg)	15.2±0.4	138*[1]	127*[3]
(10 mg/kg)	15.0±1.0	136*[1]	125*[3]
(5 mg/kg)	14.2±0.8	129*[1]	116*[3]
+ Cyclosporin A (20 mg/kg)	9.4±3.4	86	78
(10 mg/kg)	10.8±4.1	98	90
(5 mg/kg)	10.0±3.3	91	83
+ Verapamil (75 mg/kg)	13.0±0	118*[1]	108*[3]
(50 mg/kg)	13.0±0	118*[1]	108*[3]
VCR (100 μg/kg)	12.0±0	109*[1]	100
+ FK506 (20 mg/kg)	15.0±1.4	136*[2]	125*[4]
(10 mg/kg)	14.0±1.0	127*[1]	117*[4]
(5 mg/kg)	14.0±1.4	127*[1]	115*[5]
+ Cyclosporin A (20 mg/kg)	11.0±4.6	100	92
(10 mg/kg)	15.0±3.1	136*[2]	125
(5 mg/kg)	14.4±1.3	131*[1]	120*[4]
+ Verapamil (75 mg/kg)	13.0±0.7	118*[1]	108*[5]
(50 mg/kg)	13.0±0	118*[1]	108*[3]

[a] Samples of 10^6 cells of P388/VCR were implanted i. p. into each group of five CD_2F_1 mice on day 0, and drugs were given i. p. daily from days 1 to 5.

[b] T/V, at each VCR dosage, the mean survival time of the treated group divided by the mean survival time of the group of mice treated with VCR alone.

[c] Mean ± SD

[1],[2] Significant difference from control group by Student's *t*-test: *[1] $P<0.001$; *[2] $P<0.01$

[3],[4],*[5] Significant difference from VCR alone group by Student's *t*-test: *[3] $P<0.001$; *[4] $P<0.01$; *[5] $P<0.05$

CsA alone also slightly increased the life span of P388/VCR bearers. FK-506, given five times with VCR, significantly increased the life span of the P388/VCR-bearing mice. The most prominent results were observed when FK-506 (5–20 mg/kg) was administered with 200 μg/kg VCR, and T/C values were 140%–151%. At VCR doses of 150 and 100 μg/kg, T/C values of 129%–138% and 127%–136% were obtained with the combined treatment with 5–20 mg/kg FK-506. CsA at 5 mg/kg and 10 mg/kg combined with 100 μg/kg VCR resulted in the T/C values 131% and 136%, respectively. The combination of higher doses of VCR (150 and 200 μg/kg) with CsA (5–20 mg/kg) was presumably toxic to the mice and resulted in a decreased life span in comparison with control animals. Verapamil at 50 mg/kg and 75 mg/kg with 200 μg/kg VCR resulted in T/C values of 122% and 124%, respectively.

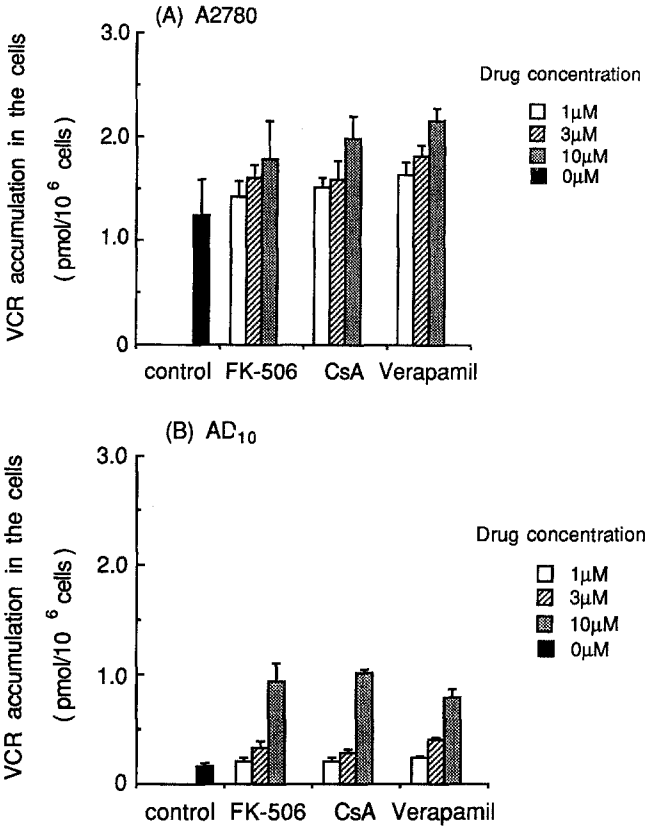

Fig. 1. The increase in vincristine (*VCR*) accumulation in the cells by FK-506. [^{3}H]VCR accumulated in A2780 (**A**) and AD_{10} (**B**) cells was determined as described in Materials and methods. Data are from triplicate determinations. *Error bars* represent SD. *CsA*, cyclosporin A.

Table 4. Inhibition of VCR binding to K562/ADM plasma membrane by FK-506[a]

Drug	IC_{50} (μM)
Vinblastine	1.1±0.2
FK-506	0.8±0.05
CsA	0.7±0.04
Verapamil	6 ±1

[a] IC_{50} values were determined as described in Materials and methods. The values represent mean ± SD of quadruplicate determinations

Effect of FK-506 on the cellular accumulation of [^{3}H]VCR

The accumulation of VCR in AD_{10} cells was greatly increased by the addition of FK-506 (Fig. 1B). AD_{10} cells (10^6 cells) accumulated 0.17 pmol VCR after incubation with 15 nM VCR at 37°C for 2 h. When FK-506 at 3 μM and 10 μM was added to the incubation mixture, the amount of VCR accumulated in the cells increased to

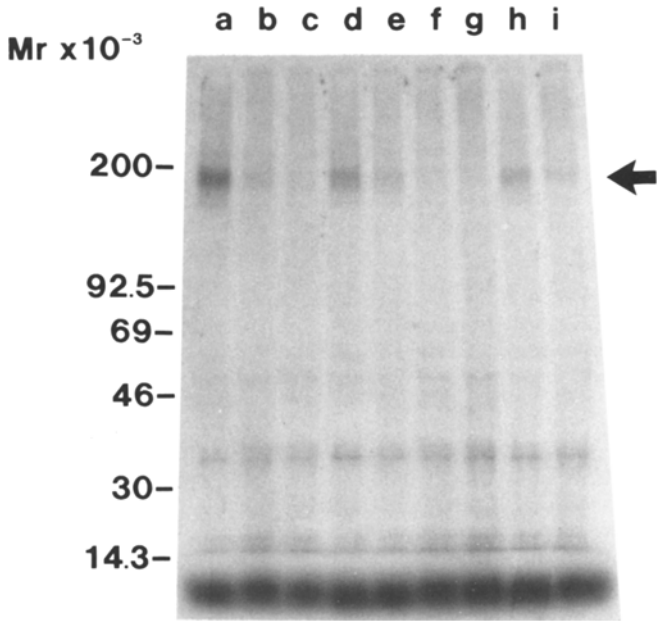

Fig. 2. Inhibiton of [^{3}H]azidopine photolabeling to P-glycoprotein by FK-506. K562/ADM membrane was photolabeled with [^{3}H]azidopine in the presence (*lanes b, d, f, h* 10 μM; *lanes c, e, g, i* 100 μM) or absence (*lane a*) of drugs as described in Materials and methods. *Lanes b, c,* FK-506; *d, e,* CsA; *f, g,* verapamil; *h, i,* VCR. The *arrow* on the right indicates P-glycoprotein

0.34 pmol and 0.94 pmol respectively. In contrast to the resistant tumor cells, A2780 cells accumulated a large amount of VCR (1.24 pmol/10^6 cells) in the absence of the modifiers, and the amount was slightly increased by the addition of FK-506 (Fig. 1A). Almost identical results were obtained by the addition of CsA and verapamil instead of FK-506.

Inhibition of drug binding by FK-506

In order to study the mechanism of reversal by FK-506, two sets of experiments were carried out using drug-binding systems. Table 4 shows the inhibitory activity of FK-506 on the binding of [^{3}H]VCR to K562/ADM plasma membrane. FK-506 as well as CsA inhibited the VCR binding to K562/ADM membrane efficiently, and the IC_{50} values of FK-506 and CsA were 0.8 μM and 0.7 μM, respectively. Verapamil was less effective in this system (IC_{50}, 6 μM). As the ATP-dependent VCR binding to K562/ADM plasma membrane closely correlates with the drug efflux from the resistant cells [7, 8], this result indicates that FK-506 as well as CsA and verapamil inhibits the VCR transport on resistant cell membranes.

Figure 2 shows the inhibitory activity of FK-506 on the binding of [^{3}H]azidopine to P-glycoprotein. FK-506 and verapamil at 10 μM and 100 μM almost completely inhibited the binding of azidopine to P-glycoprotein. CsA and VCR at 10 μM partially inhibited the azidopine binding, and at 100 μM the binding was inhibited almost completely. This result indicates that FK-506 directly interacts with P-glycoprotein like VCR, verapamil and CsA.

Discussion

FK-506 is a novel immunosuppressive agent with macrolide structure [16]. It is more potent than CsA in various immunosuppressive activities and in suppression of the rejection of transplants [4–6]. We found that FK-506 was also effective in overcoming multidrug resistance as well as CsA. FK-506 enhanced the cytotoxicity of VCR in AD_{10}, K562/ADM and P388/VCR cells, and of ADM in AD_{10} cells in vitro. The resistance to VCR in moderately resistant P388/VCR cells (40-fold resistance) was completely reversed by 3 μM FK-506, which was an almost nontoxic dose of FK-506 in this cell line. This result indicates that FK-506 is effective in overcoming a lower degree of multidrug resistance in vitro. The resistance in AD_{10} and K562/ADM cells, however, was not completely overcome by FK-506 at 10 μM. These cell lines were highly resistant against VCR (AD_{10}, 1150-fold; K562/ADM, 850-fold), and a complete reversal could not be achieved by either CsA or verapamil. These highly resistant cell lines might have another mechanism of resistance, which these modifiers could not affect.

FK-506 is also effective in overcoming VCR resistance in vivo. The chemotherapeutic effect of VCR in P388/VCR-bearing mice was enhanced to 151% (T/C) when 20 mg/kg of FK-506 was combined with 200 μ/kg of VCR. At doses of 200 and 150 μg/kg of VCR, the maximum chemotherapeutic effect combined with FK-506 was significantly superior to the effect of other combinations (P <0.001 by Student's t-test).

FK-506 inhibited the [^{3}H] azidopine binding to P-glycoprotein efficiently. The VCR binding to K562/ADM plasma membrane was inhibited by FK-506 as effectively as by CsA. Furthermore, the accumulation of VCR in AD_{10} cells was increased by FK-506 as effectively as by CsA and verapamil. These results strongly suggest that the mechanism of action of FK-506 for overcoming multidrug resistance is similar to that of CsA and verapamil. It was reported that verapamil competitively bound to the drug-binding site on P-glycoprotein and was transported from resistant cells by a mechanism similar to that of antitumor agents [8, 26]. CsA is also regarded as a competitive inhibitor for the transport of antitumor agents by P-glycoprotein [8, 13]. Assuming that FK-506 is also recognized by P-glycoprotein and transported out from resistant cells, FK-506 could be effective in accumulating VCR in resistant cells and in overcoming multidrug resistance. In addition to the common effect of these compounds on P-glycoprotein, FK-506, CsA and verapamil might have another individual mechanism of action yet to be elucidated on each multidrug-resistant cell, because the relative potency of FK-506, CsA and verapamil to reverse resistance is different in AD_{10}, K562/ADM and P388/VCR cells in vitro.

The dosage of FK-506 for overcoming of resistance in P388/VCR-bearing mice is rather high as compared to the dose for suppression of the rejection of transplants [4]. This immuno-suppressive activity might restrict the clinical use of FK-506 for overcoming of drug resistance. It is not understood whether the overcoming activity of the drug resistance by FK-506 correlates with its immunosuppressive activity. Regarding the direct interaction of FK-

506 with P-glycoprotein, however, the reversing activity is not likely to correlate with the immunosuppressive activity. Actually, in the case of cyclosporins, non-immunosuppressive cyclosporins can modify the drug resistance as well as CsA [25]. Non-immunosuppressive FK-506 analogues might be more effective than FK-506 in overcoming of multidrug resistance, and the search for such compounds would be of interest.

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