Anti-Cancer Drugs 1996, 7, pp. 825–832

Cremophor EL reversed multidrug resistance *in vitro* but not in tumor-bearing mouse models

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Cremophor EL (CreEL), a polyethylene castor oil used as a vehicle for cyclosporin A and taxol, reverses P-glycoprotein-mediated drug resistance. The vehicle in an i.v. dosage form of PSC 833, [3′-keto-Bmt[1]]-[Val[2]]-cyclosporin, contains CreEL and has been presumed to have the potentiation of the reversal activity of PSC 833. To examine this possibility, we compared reversal activities of CreEL and PSC 833 against multidrug resistance (MDR) *in vitro* and *in vivo*. Both CreEL and PSC 833 inhibited P-glycoprotein-mediated efflux of [^{3}H]vincristine from adriamycin-resistant myelogenous leukemia K562. The sensitization of multidrug-resistant cell lines to anticancer drugs by CreEL and PSC 833 was selective to MDR-related agents, suggesting a specific interference of the P-glycoprotein function by the two MDR modulators. The concentration-dependent activity of the modulators demonstrated that CreEL is at least 100 times less potent than PSC 833. The *in vivo* reversal effects of CreEL alone and PSC 833 in the vehicle were investigated in multidrug-resistant tumor-bearing mouse models. In vincristine-resistant P388 leukemia-bearing mice, neither i.v. nor i.p. administration of CreEL even at 1440 mg/kg enhanced the antitumor activity of adriamycin. The *in vivo* negligible activity of CreEL was confirmed in an HCT-15-bearing athymic mouse model. In contrast, PSC 833 significantly enhanced the antitumor activity of adriamycin in the *in vivo* models. The reversal activity of CreEL restricted to *in vitro* leads us to conclude that the vehicle containing CreEL did not potentiate the activity of PSC 833 in the tumor-bearing mouse models.

Key words: Cremophor EL, multidrug resistance, tumor-bearing mice, P-glycoprotein, PSC 833.

Introduction

One of the major obstacles in cancer chemotherapy is the development of drug resistance during treatment. Enhanced metabolic inactivation and/or the excretion of anticancer drugs in patients may contribute to clinical resistance to anticancer drugs. The hypothetical mechanisms for developing a resistant tumor cell population are: (i) the heterogeneity of drug sensitivity in tumor cell populations, (ii) the inherent resistance of some types of tumors having marginal response rates to cancer chemotherapy, e.g. colorectal, renal and gastric cancer, and (iii) acquired resistance following treatment.[1,2] When tumor cells acquire resistance to anticancer drugs such as vinca alkaloids and anthracyclines, they often show cross-resistance to a number of anticancer drugs having different structures and modes of action [multidrug resistance (MDR)].[1] P-glycoprotein, a member of the ATP binding cassette transporter, plays a major role in the so-called classical multidrug-resistant phenotype.[1,2] Functioning as an ATP-hydrolyzing efflux pump for a number of anticancer agents, P-glycoprotein causes a reduction in the intracellular concentration of the MDR-related anticancer drugs.[2]

A strategy for overcoming classical MDR has been developed since the identification of the P-glycoprotein inhibitor verapamil.[3] The biomodulation of P-glycoprotein function by verapamil significantly improved the antitumor activity of vinca alkaloids and anthracyclines against multidrug-resistant tumors.[3] Verapamil directly binds to P-glycoprotein and inhibits drug efflux by being transported itself.[4] In the last decade, a number of the P-glycoprotein inhibitors have been identified, e.g. calcium channel blockers,[5,6] immunosuppressants cyclosporin A[7,8] and FK506,[9] and surfactant Cremophor EL (CreEL).[10–13] CreEL, a polyethoxylated castor oil, is used clinically as a vehicle for the hydrophobic drugs taxol (TAX) and cyclosporin A. This surfactant has also been used in galenical form for i.v. administration of [3′-keto-Bmt[1]]-[Val[2]]-cyclosporin (PSC 833), a cyclosporin D derivative able to modulate MDR.[14–18] The vehicle has been presumed to show

Supported by a special grant for Advanced Research on Cancer from the Ministry of Education, Science and Culture, Japan. The first two authors contributed equally to this work.

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the potentiation of the reversal activity of PSC 833. To examine this possibility, we compared the reversal effects of CreEL with those of PSC 833 on: (i) the efflux of vincristine from P-glycoprotein-overexpressing cells, (ii) sensitivity to anticancer drugs *in vitro* and (iii) the antitumor activity of adriamycin (ADM) in multidrug-resistant tumor-bearing mouse models.

Materials and methods

Chemicals

A galenical form of PSC 833 for i.v. injection containing CreEL and EtOH (PSC 833, 50 mg, CreEL, 600 mg; EtOH, remainder per 1 ml solution), and its placebo were supplied by Sandoz Pharmaceuticals (Basel, Switzerland). ADM for clinical usage was purchased from Kyowa Hakko Kogyo (Tokyo, Japan) and mitoxantrone (MIT) from Lederle Japan (Tokyo, Japan). TAX, etoposide (VP16), 5-fluorouracil (5-FU) and cytosine β-D-arabino-fluoroside (AraC) were purchased from Sigma (St Louis, MO). [G-^{3}H]Vincristine ([^{3}H]vincristine, 7.0 Ci/mmol) was purchased from Amersham Japan (Tokyo, Japan). All other chemicals were purchased commercially and were of analytical grade. For animal treatments, CreEL and PSC 833 were diluted with 5% glucose, and ADM with saline.

Animals and tumor cells

Six-week-old female BALB/c *nu/nu* and BALB/c × DBA/2 (CDF_1) mice were purchased from Clea Japan (Tokyo, Japan) and Charles River Japan (Kanagawa, Japan), respectively. Animal studies were performed under the guidelines of the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, and Tsukuba Research Institute, Sandoz Pharmaceuticals. P-glycoprotein overexpressing cell lines and the following parental cell lines used were: (i) a vincristine-resistant P388 murine leukemia, P388/VCR, kindly supplied by the National Cancer Institute (NIH, Bethesda, MD); (ii) the human myelogenous leukemia K562, kindly provided by Dr K Ezaki (Cancer Chemotherapy Center, Japan) and its adriamycin-resistant subline K562/ADM, established in our laboratory;[19] (iii) the human ovarian carcinoma A2780 and its adriamycin-resistant subline AD10, provided by Drs Ozols and Hamilton (Medicine Branch, National Cancer Institute, Bethesda, MD); (iv) a cloned human epidermal carcinoma KB3-1 and its colchicine-resistant subline KBC-4, provided by Dr Pastan (National Cancer Institute, Bethesda, MD). Human colorectal adenocarcinoma HCT-15 (CCL 225) was obtained from ATCC (Rockville, MD), through Dainippon Pharmaceutical (Osaka, Japan). The human tumor cells were maintained in RPMI 1640 (Nissui, Tokyo, Japan), supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine and 100 μg/ml kanamycin in 5% CO_2 at 37°C. P388/VCR was passaged by weekly transfer of 10^6 cells into the peritoneal cavity of CDF_1 mice.[9]

Efflux experiment

The inhibitory effects of CreEL or PSC 833 on the efflux of vincristine from cells were evaluated, as described previously.[20] In brief, K562 and K562/ADM cells were initially suspended at a density of 2×10^6 cells/ml in glucose-free Hanks' balanced salt solution (HBSS) supplemented with 10 mM NaN_3 and 10 mM HEPES (loading medium). Cells were incubated with [^{3}H]vincristine with or without the modulator at 37°C for 30 min in loading medium to reach a sufficient intracellular level of [^{3}H]vincristine. After washing twice with ice-cold PBS, drug efflux was initiated by resuspending the cells in NaN_3-free and glucose-containing HBSS prewarmed at 37°C in the presence or absence of the modulator at the same concentrations as before washing. After the given time, cells were centrifuged and washed twice with ice-cold PBS. The retained radioactivity was determined using a liquid scintillation counter model LSZ-355 (Beckman, Tokyo, Japan). The radioactivity at each point is represented as the percentage of radioactivity at time 0 for each group.

Evaluation of reversal efficacy in growth inhibition *in vitro*

The growth inhibition of the tumor cells by anticancer drugs was determined by the 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) assay according to a method described previously.[21,22] Tumor cells in the phenol-red-free growth medium were inoculated in 96-well microplates at a density of 5×10^3 cells/well for AD10 cells and 2×10^3 cells/well for K562/ADM, K562, KBC-4, KB3-1 and A2780 cells. The cells were preincubated at 37°C for 24 h for the adherent cells, and 3 h for the K562/ADM and K562 cells. The anticancer drug and the modulator were added to each well and this was followed by further incubation for 72 h. Then 50 μl of the XTT solution was added to each well. After

incubation at 37°C for 4 h, the optical density was measured at 450 nm. The mean concentration of anticancer drug resulting in a 50% inhibition of the growth of the control (IC_{50}) was determined by plotting the logarithm of the drug concentration against the growth rate. The sensitization activity of the modulator was expressed as a dose-modifying factor, a ratio of the IC_{50} of the control group to that in the presence of the modulator.

Evaluation of reversal efficacy of the MDR modulator in tumor-bearing mouse models

The reversal efficacy of CreEL and PSC 833 in tumor-bearing mouse models was evaluated as described previously.[17,18,23] P388/VCR cells (10^6) were inoculated i.p. in the CDF_1 mice on day 0. The P388/VCR-bearing mice were treated with ADM, CreEL, PSC 833 or combinations of these on days 1, 5 and 9. CreEL or PSC 833 was administered 1 h prior to the treatment with ADM. Antitumor activity was evaluated based on: (i) mean survival time of the drug-treated mouse group (T) divided by the mean survival time of the control group (C) [T/C (%)] and (ii) mean survival time of the modulator-pretreated mouse group (T) divided by the mean survival time of the group treated with ADM alone (A) [T/A (%)].[18] Antitumor activity in an established HCT-15 xenograft model was evaluated as described previously.[18,23] HCT-15 cells (10^6) in 0.1 ml of HBSS was inoculated into the right subaxillary region of athymic mice. The control group consisted of 10 mice and the treated group of five mice. Drug treatment was initiated after establishment of the tumor tissue reached 100–500 mm^3 (day 0), as estimated by caliper measurement. The modulators and ADM were given i.v. on day 1. The long and short diameters of tumor tissue were measured using calipers until 30 days after the treatment. The tumor volume was calculated from the equation:

$$V = 1/2 \times a \times b^2$$

where V is the tumor volume, and a and b are the long and short diameters of the tumor mass (in mm). Antitumor activity was evaluated based on tumor growth inhibition.[18,23] The therapeutic regimen in the tumor-bearing mouse models is given in Table 1.

Results

Inhibition of the efflux of [^{3}H]vincristine from K562/ADM by CreEL and PSC 833

K562/ADM cells were suspended in glucose-free and NaN_3-containing HBSS and preloaded with [^{3}H]vincristine to accumulate a sufficient level of the substrate, since overexpression of P-glycoprotein kept the accumulated level of [^{3}H]vincristine very low under normal conditions (data not shown). The P-glycoprotein-mediated efflux was initiated by suspending the cells in normal HBSS. K562/ADM resuspended in normal HBSS excreted [^{3}H]vincristine much faster than K562 (Figure 1). CreEL and PSC 833 inhibited the [^{3}H]vincristine efflux from K562/ADM in a concentration-dependent manner. CreEL partially inhibited the efflux of [^{3}H]vincristine at concentrations lower than 100 μg/ml and completely at 300 μg/ml. PSC 833 inhibited the efflux partially at 0.3 μg/ml and completely at 1 μg/ml.

Reversal effect on the sensitivity of cells *in vitro*

The cytotoxic activity of several anticancer drugs with and without modulators was examined by the XTT assay. First, effects of the modulators on

Table 1. Treatment regimen in the tumor-bearing mice models

Table or figure	Tumor	Schedule	Antitumor drug		Modulator	
				Dose		Dose
Table 2	P388/VCR	days 1, 5, 9	ADM	2–6 mg/kg i.v.	CreEL	720, 1440 mg/kg i.p., i.v.
Table 3	P388/VCR	days 1, 5, 9	ADM	2–6 mg/kg i.p.	CreEL	720, 1440 mg/kg i.p., i.v.
Table 4	P388/VCR	days 1, 5, 9	ADM	2–6 mg/kg i.v.	PSC 833[a]	7.5, 15, 30 mg/kg i.v.
Figure 4	HCT-15	day 1	ADM	10 mg/kg i.v.	CreEL PSC 833[a]	360 mg/kg i.v. 15 mg/kg i.v.

[a]Each dosing solution of PSC 833 contained CreEL at a dosage of 360 mg/kg.

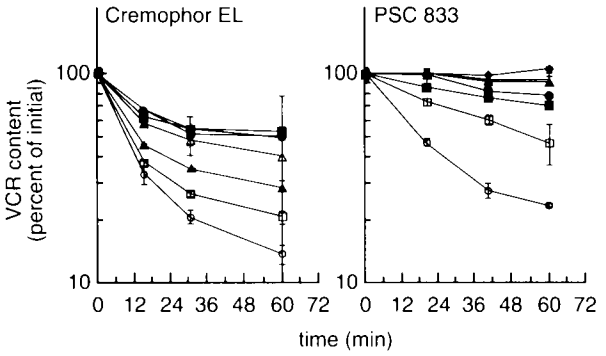

Figure 1. Effect of CreEL and PSC 833 on efflux of [^{3}H]vincristine from K562/ADM cells. K562 and K562/ADM cells were preloaded with [^{3}H]vincristine. After washing with ice-cold PBS, the efflux was initiated by resuspending K562 (●) and K562/ADM (○, ■, □, ▲, △, ◆) in [^{3}H]vincristine-free HBSS. The medium conditions were normal HBSS without the modulator (●, ○), glucose-free HBSS with 10 mM NaN_3 (■), or normal HBSS with CreEL at 10 (□), 30 (▲), 100 (△) or 300 (◆) μg/ml in the left panel, or with PSC 833 at 0.3 (□), 1 (▲), 3 (△) or 10 (◆) μg/ml in the right panel. Each point and bar represents the mean and SD of triplicate determinations, respectively.

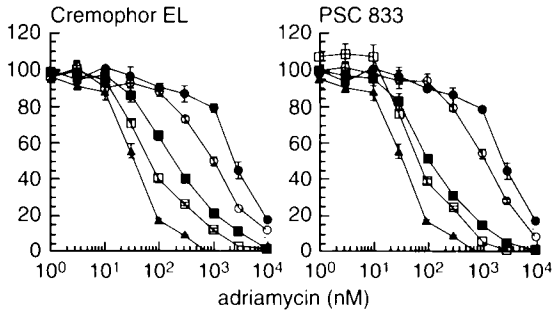

Figure 2. Effects of CreEL and PSC 833 on growth-inhibitory actions of ADM on K562 and K562/ADM cells *in vitro*. K562 (▲) and K562/ADM (●, ○, ■, □) were exposed to graded concentrations of ADM with or without the modulator for 72 h. The growth inhibition by the treatments were evaluated by the XTT assay, as described in Materials and methods. The modulator concentrations used were: CreEL 0 (●), 10 (○), 30 (■) or 100 (□) μg/ml in the left panel; and PSC 833 at 0 (●), 0.3 (○), 1 (■) or 3 (□) μg/ml in the right panel. Each point and bar denotes the mean and SD of triplicate experiments, respectively.

antitumor activity of ADM were investigated in K562/ADM (Figures 2 and 3). Both modulators enhanced the cytotoxicity of ADM in K562/ADM, but not in K562, in a concentration-dependent manner. PSC 833 was suggested to be approximately 100 times more potent than CreEL, since the activity of CreEL at 300 μg/ml was comparable to that of PSC 833 at 3.6 μg/ml (Figures 2 and 3). The difference in potency between PSC 833 and CreEL in the growth inhibition assay was consistent with that in the efflux assay. Next, we examined effects of the modulators on sensitivity of K562 and K562/ADM against various antitumor drugs *in vitro* (Figure 3). The anticancer drugs included MDR-related drugs of ADM, MIT, TAX and VP16, and non-MDR-related drugs of AraC and 5-FU. The sensitization activity of the modulator was expressed as a dose-modifying factor, a ratio of the IC_{50} of the control group to that in the presence of the modulator (Figure 3). The sensitization of the multidrug-resistant cells by CreEL and PSC 833 was selective to the MDR-related anticancer drugs ADM, TAX, MIT and VP16, since the modulators minimally affect the sensitivity of K562/ADM to AraC and 5-FU. In addition, the modulators did not markedly sensitize the drug-sensitive K562. The effects of the modulators on the sensitivity of A2780, KB3-1 and these multidrug-resistant cells against MIT, VP16 and AraC were consistent with the effects in the sublines of K562.

Reversal activity of CreEL and PSC 833 in tumor-bearing mouse models

The reversal effect of CreEL was evaluated in a P388/VCR-bearing mouse model (Tables 2 and 3). Neither i.v. nor i.p. pretreatment with CreEL enhanced the antitumor activity of ADM given i.v., since the maximum T/A value was 122% (Table 2). When ADM was administered i.p., it alone significantly increased the life span of the mice (Table 3). The T/C value was 152% at a 2 mg/kg dose of ADM, 184% at 4 mg/kg and 193% at 6 mg/kg. Pretreatment with CreEL produced only a negligible effect on the antitumor activity of the i.p. treatment with ADM and the maximum T/A value was 118% at a 1440 mg/kg i.p. dose of CreEL in combination with 2 mg/kg i.p. of ADM. On the other hand, PSC 833 in the vehicle significantly enhanced the antitumor activity of ADM even given i.v. (Table 4). The i.v. treatment with 30 mg/kg of PSC 833 in combination with i.v. dosings of ADM at 3 mg/kg produced a maximum T/C value of 199% (T/A, 193%).

The effect of CreEL and PSC 833 on the antitumor activity of ADM was evaluated in an HCT-15-bearing athymic mouse model. The established xenograft of HCT-15 were treated with ADM with or without the modulator on day 1 (Figure 4). The i.v. treatment with ADM at 10 mg/kg on day 1 slightly inhibited the growth of that tumor by approximately 40% on day 15. The pretreatment with i.v. dosing of PSC 833 at 15 mg/kg resulted in a 70% growth inhibition,

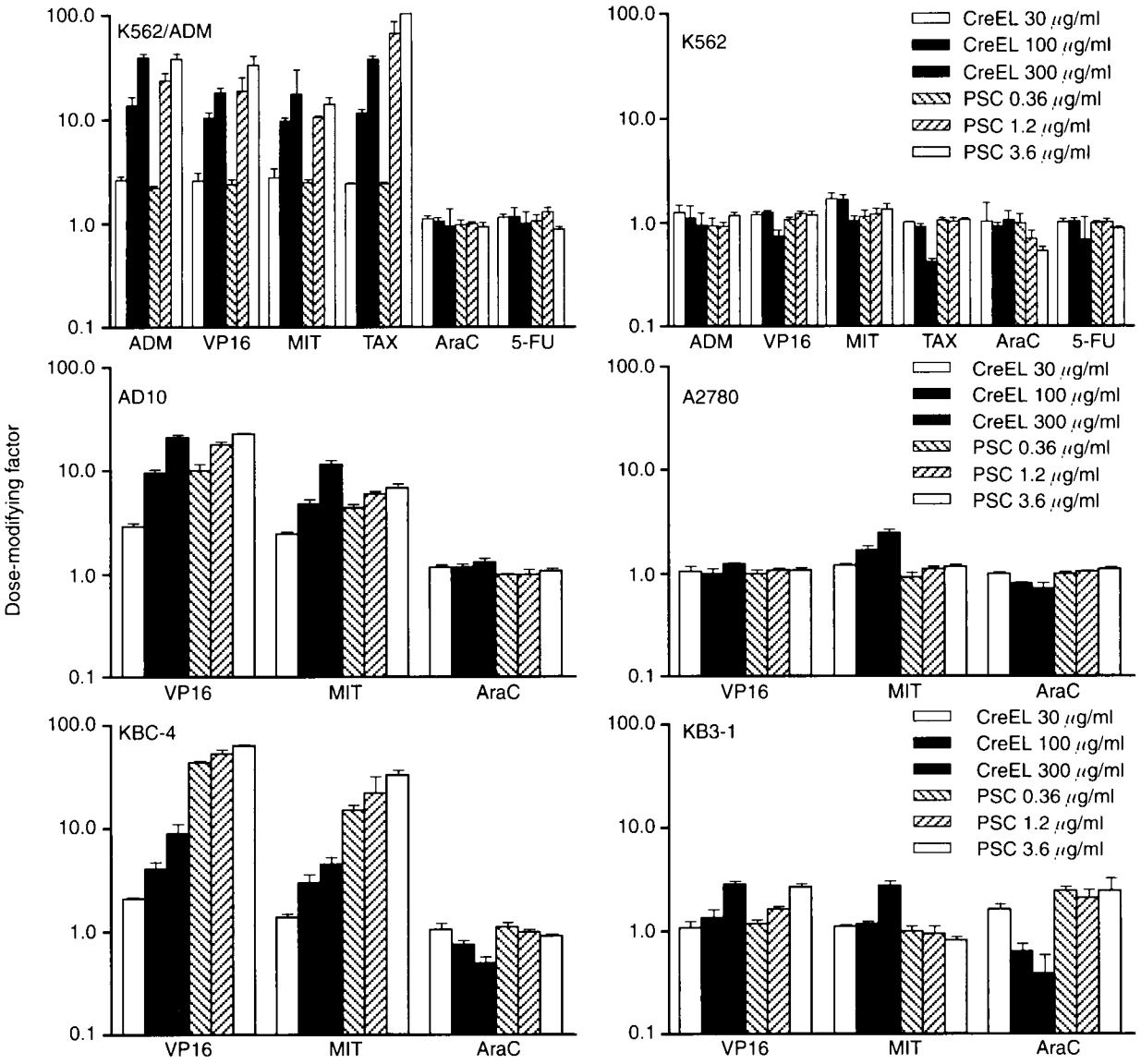

Figure 3. Sensitization by CreEL and PSC 833 of multidrug-resistant and sensitive cells against anticancer drugs. Multidrug-resistant and sensitive cells were exposed with graded concentrations of ADM with or without the modulator for 72 h. The growth inhibition by the treatments was evaluated by the XTT assay, as described in Materials and methods. The modulator concentrations used are indicated in the right panels. The activity of the modulators was indicated by the dose-modifying factor, the ratio of IC_{50} of the control to IC_{50} of the cells with the modulator. Data represents the mean and SD of triplicated experiments.

although the CreEL pretreatment at 360 mg/kg i.v. resulted in only a 30% inhibition. These results indicate that the CreEL at a dose of 360 mg/kg was inactive in sensitizing the HCT-15 xenograft against ADM.

As a result, the pretreatment with CreEL did not enhance the antitumor activity of ADM in the tumor-bearing mouse models, while PSC 833 in the vehicle produced significant enhancement of the antitumor activity of ADM.

Discussion

In the last decade, a considerable number of MDR reversing agents have been identified. These agents commonly inhibit the functions of the MDR1 gene product P-glycoprotein. Previous studies have demonstrated that both PSC 833 and CreEL also competed azidopine binding to P-glycoprotein,[12,25] suggesting that both compounds inhibited the P-glycoprotein function by direct binding near the

Table 2. Effect of CreEL on antitumor activity of ADM (i.v. treatment) in P388/VCR-bearing mice

ADM [mg/kg (i.v.)]	CreEL Route	CreEL (mg/kg)	T/C[a] (%)	T/A[b] (%)	MST[c] ± SD (day)
0	i.p.	5% glucose	100		12.0 ± 0.00
	i.p.	720	97		11.7 ± 1.15
	i.p.	1440	100		12.0 ± 0.00
	i.v.	720	100		12.0 ± 1.00
	i.v.	1440	97		11.7 ± 0.58
2	i.p.	5% glucose	100	100	12.0 ± 1.00
	i.p.	720	103	103	12.3 ± 0.58
	i.p.	1440	106	106	12.7 ± 1.15
	i.v.	720	100	100	12.0 ± 1.00
	i.v.	1440	92	92	11.0 ± 0.00
4	i.p.	5% glucose	100	100	12.0 ± 0.00
	i.p.	720	122	122	14.7 ± 2.08
	i.p.	1440	111	111	13.3 ± 2.31
	i.v.	720	117	117	14.0 ± 2.65
	i.v.	1440	100	100	12.0 ± 1.41
6	i.p.	5% glucose	108	100	13.0 ± 1.00
	i.p.	720	111	103	13.3 ± 0.58
	i.p.	1440	119	110	14.3 ± 1.53
	i.v.	720	114	110	13.7 ± 0.58
	i.v.	1440	100	92	12.0 ± 1.73

P388/VCR cell (10^6) were inoculated i.p. in CDF_1 mice on day 0. Mice were treated on days 1, 5 and 9 with CreEL or its placebo (5% glucose solution) i.v. or i.p. 1 h prior to i.v. treatment with adriamycin. Each group consisted of three mice.
[a]Mean survival time of drug-treated mouse group divided by mean survival time of control group.
[b]Mean survival time of modulator-pretreated mouse group divided by mean survival time of anticancer drug alone-treated mouse group.
[c]Mean survival time in days.

Table 3. Effect of CreEL on antitumor activity of ADM (i.p. treatment) in P388/VCR-bearing mice

ADM [mg/kg (i.v.)]	CreEL Route	CreEL (mg/kg)	T/C[a] (%)	T/A[b] (%)	MST[c] ± SD (day)
0	i.p.	5% glucose	100		11.0 ± 0.82
	i.p.	720	98		10.8 ± 0.50
	i.p.	1440	107		11.8 ± 0.96
	i.v.	720	107		11.8 ± 1.26
	i.v.	1440	102		11.3 ± 1.50
2	i.p.	5% glucose	152	100	16.8 ± 0.96
	i.p.	720	166	109	18.3 ± 0.50*
	i.p.	1440	180	118	19.8 ± 2.06*
	i.v.	720	150	99	16.5 ± 0.58
	i.v.	1440	159	105	17.5 ± 0.58
4	i.p.	5% glucose	184	100	20.3 ± 2.22
	i.p.	720	186	101	20.5 ± 2.38
	i.p.	1440	200	109	22.0 ± 2.94
	i.v.	720	184	100	20.3 ± 1.50
	i.v.	1440	193	105	21.3 ± 3.69
6	i.p.	5% glucose	193	100	21.3 ± 2.06
	i.p.	720	175	91	19.3 ± 2.06
	i.p.	1440	180	93	19.8 ± 2.22
	i.v.	720	184	95	20.3 ± 3.77
	i.v.	1440	198	102	21.8 ± 2.99

P388/VCR cells (10^6) were inoculated i.p. in CDF_1 mice on day 0. Mice were treated on days 1, 5 and 9 with CreEL or its placebo (5% glucose solution) i.v. or i.p. 1 h prior to i.v. treatment with adriamycin. Each group consisted of three mice.
[a]Mean survival time of drug-treated mouse group divided by mean survival time of control group.
[b]Mean survival time of modulator-pretreated mouse group divided by mean survival time of anticancer drug alone-treated mouse group.
[c]Mean survival time in days.
*Significant difference from the ADM-only group by Mann Whitney's *U*-test ($p < 0.05$).

substrate sites. The present study indicated that the activity of both modulators in sensitizing tumor cells was selective to P-glycoprotein-positive cells, since they did not markedly sensitize the parental cell lines *in vitro*. In addition, the sensitization of P-glycoprotein-positive cell lines to anticancer drugs by CreEL and by PSC 833 was specific to MDR-related agents (Figure 3). These findings supported the conclusion that sensitization of multidrug-resistant cells by CreEL and PSC 833 was attributed to interaction of CreEL and PSC 833 with P-glycoprotein.

CreEL has been used clinically as a vehicle for the hydrophobic drugs TAX and cyclosporin A. This surfactant has also been used in galenical form for i.v. administration of PSC 833. To examine the possibility that CreEL in the galenical form of PSC 833 enhanced the reversal activity of PSC 833, we compared the dose intensity of CreEL and PSC 833 *in vitro* and *in vivo*. The modulator both inhibited the vincristine efflux and sensitized the MDR cells in a concentration-dependent manner (Figure 1). Concentrations of CreEL and PSC 833 required to completely inhibit the efflux were 300 and 1 μg/ml, respectively. Thus PSC 833 was suggested to be approximately 100 times more potent than CreEL. The greater potency of PSC 833 compared to CreEL was confirmed by the *in vitro* growth inhibition assay.

Next we compared *in vivo* activities of CreEL and PSC 833 in a vehicle containing CreEL. The present study demonstrated that CreEL even at 1440 mg/kg did not potentiate the antitumor activity of ADM in P388/VCR-bearing mice. In addition, the minimal activity of CreEL was confirmed in an HCT-15-bearing athymic mouse model. In contrast, PSC 833 significantly sensitized the P-glycoprotein-positive tumors. The negligible activity of CreEL in the

Table 4. Effect of PSC 833 on antitumor activity of ADM in P388/VCR bearing mice

ADM [mg/kg (i.v.)]	PSC 833 [mg/kg (i.v.)]	T/C[a] (%)	T/A[b] (%)	MST[c] ± SD (day)
0	5% glucose	100		12.0 ± 0.7
	30	110		13.2 ± 2.5
2	0	100	100	12.0 ± 0.9
	30	157	157	18.8 ± 1.0*,***
3	0	103	100	12.3 ± 1.0*
	15	169	165	20.3 ± 1.9*,***
	30	199	193	23.8 ± 1.2*,***
4	0	108	100	13.0 ± 0.9*
	7.5	167	154	20.0 ± 1.3*,***
	15	197	182	23.7 ± 2.6*,***
	30	181	167	21.7 ± 6.7*,***
5	0	103	100	12.3 ± 0.5
	7.5	185	180	22.2 ± 2.5*,***
	15	185	180	22.2 ± 8.0
6	0	113	100	13.5 ± 1.1**
	7.5	169	151	20.3 ± 6.4

P388/VCR cells (10^6) were inoculated i.p. in CDF_1 mice on day 0. Mice were treated on days 1, 5 and 9 with PSC 833 or its placebo (360 mg/kg CreEL) i.v. 1 h prior to i.v. treatment with ADM. The control group consisted of 10 mice and the drug-treated group consisted of six mice.

[a]Mean survival time of drug-treated mouse group divided by mean survival time of control group.

[b]Mean survival time of modulator-pretreated mouse group divided by mean survival time of anticancer drug alone-treated mouse group.

[c]Mean survival time in days.

Significant difference from control group by Mann Whitney's *U*-test: * $p < 0.001$, ** $p < 0.01$.

***Significant difference from ADM-only group by Mann Whitney's *U*-test ($p < 0.01$).

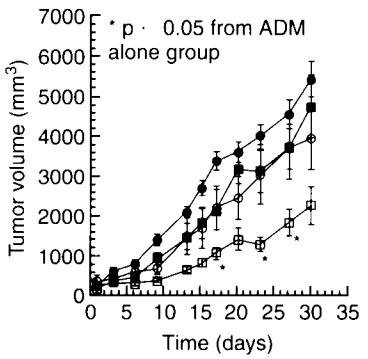

Figure 4. Effect of CreEL and PSC 833 on antitumor activity of ADM in athymic mice bearing the HCT-15 tumor. The volumes of HCT-15 xenografts reached 100 500 mm^3 8 days after tumor inoculation (day 0). On day 1, the mice were pretreated with glucose solution (●, ○), CreEL (360 mg/kg, i.v., ■) or PSC 833 (15 mg/kg, i.v., □). At 1 h after pretreatment, ADM (10 mg/kg, ○, ■, □) or saline (●) was given i.v. Each point and bar denotes the mean and SE, respectively, of at least five tumors.

tumor-bearing mouse models suggested that the *in vivo* reversal activity of PSC 833 in the vehicle was not attributed to CreEL.

In tumor-bearing mouse models, several MDR-reversing agents were found to be inactive in potentiating antitumor activity of MDR-related agents, although they are active *in vitro*.[21] CreEL might be included in this type of agent having reversal potency only in *in vitro* models. However, Woodcock reported that CreEL enhanced the activity of ADM in ADM-resistant P388-bearing mice.[11] They found that CreEL at 1200 mg/kg *per se* increased the life span of P388/ADM-bearing mice, suggesting that the potentiation of ADM activity by CreEL was attributed to the additive cytotoxic effect of the agents. In our experiment, however, CreEL at 1440 mg/kg *per se* did not prolong the life span of the tumor-bearing mice. One may suggest that the discrepancy in the potency of CreEL *in vitro* and *in vivo* in this study was attributed to a lower available plasma concentration of CreEL than the effective concentration *in vitro*.

Webster reported that the concentrations of CreEL measured in plasma from patients after a 3 h infusion of TAX was sufficient to inhibit P-glycoprotein-mediated transport *ex vivo*.[25] In this bioassay, the sampling was made at a maximum concentration of CreEL immediately after the infusion of TAX. To assess the effectiveness of CreEL in patients, it should be noted how long the active concentrations of CreEL can be maintained.

Conclusion

CreEL and PSC 833 clearly inhibited the P-glycoprotein-mediated efflux of vincristine and sensitized the multidrug-resistant cells to MDR-related anticancer drugs. The concentration-dependent activity of the modulators demonstrated that CreEL was less effective than PSC 833 *in vitro*. CreEL did not markedly enhance the antitumor activity of ADM in tumor-bearing mouse models, while PSC 833 did. The differential activities between CreEL and PSC 833 *in vivo* suggested that CreEL in the vehicle for PSC 833 did not potentiate the activity of PSC 833 in the tumor-bearing mouse models.

Acknowledgments

We thank Dr D Cohen for her critical reading of our manuscript.

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(Received 22 August 1996; accepted in revised form 8 October 1996)