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Overcoming of multidrug resistance by VA-033, a novel derivative of apovincaminic acid ester

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Abstract

We have studied the effects of a novel derivative of apovincaminic acid ester, VA-033, on the resistance of tumors to chemotherapeutic agents. VA-033 increased the sensitivity of drug-resistant cell lines (P388/VCR, P388/ADM, AD10, and K562/ADM) to adriamycin or vincristine. The potency of VA-033 was stronger than verapamil. The drug lengthened the survival time of the P388/VCR-implanted mice treated with vincristine. VA-033 increased the intracellular accumulation of vincristine in the tumor cells, and the photolabeling of P-glycoprotein by [^{3}H]azidopine was inhibited by VA-033. VA-033 showed a slight inhibitory effect on the L-type Ca^{2+} current in the ventricular myocytes, and had less effect on the cardiovascular parameters such as blood pressure, contractile force and atrio-ventricular conduction time than verapamil when administered systemically in the dog. These results suggest that VA-033 may become a beneficial compound as a modifier to the neoplastic cell resistant to multidrugs.

Keywords: Chemotherapeutic; P-glycoprotein; Ca^{2+} current; Verapamil

1. Introduction

The resistance of tumors to many chemotherapeutic agents is well known, with the problem remaining a major impediment to cancer chemotherapy (Biedler and Riehm, 1970). It has become evident that overexpression of a M_r 170 000 transmembrane cell line called P-glycoprotein is observed in the plasma membrane of multidrug-resistant cell lines, and that P-glycoprotein functions as an energy-dependent efflux pump to chemotherapeutic agents, resulting in a decreased concentration of chemotherapeutics in neoplastic cells. As a consequence, resistance to the drugs often arises (Chen et al., 1986; Gerlach et al., 1986; Gros et al., 1986; Hamada and Tsuruo, 1988). Various agents, which were observed to attenuate resistance of tumors in animal studies, have been shown to inhibit the efflux of chemotherapeutics competitively by binding P-glycoprotein, thereby increasing the accumulation of chemotherapeutics in tumor cells (Cornwell et al., 1987; Akiyama et al., 1988). One of the most typical agents is verapamil, a Ca^{2+} channel antagonist. Verapamil causes depressant actions on the cardiac tissues and blood vessels by inhibiting transmembrane Ca^{2+} entry through Ca^{2+} channels in the cell membrane (Fleckenstein, 1983). There have been several reports showing that verapamil overcomes the resistance of tumors to chemotherapeutic agents since the study performed by Tsuruo et al. (1981). Clinical trials using verapamil showed successful results. However, a decrease in blood pressure and atrio-ventricular block in the heart were observed because verapamil exerts strong Ca^{2+} antagonistic effects on peripheral vessels and cardiac tissues (Dalton et al., 1989). In the present study, we have investigated the effects on the resistance of tumors to chemotherapeutic agents of a novel derivative of apovincaminic acid ester, VA-033. VA-033 was found to exert attenuating effects on the resistance of tumors, with only a slight effect on the cardiovascular functions.

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2. Materials and methods

2.1. Cell lines and culture

The P388 cell line and its vincristine (VCR)-resistant (P388/VCR) and adriamycin (ADM)-resistant A2780 (AD10) sublines were provided by Dr. T. Tashiro, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research (Tokyo, Japan). The ADM-resistant K562 (K562/ADM) subline was prepared by Dr. T. Tsuruo, Institute of Molecular and Cellular Biosciences, University of Tokyo (Tokyo, Japan). The ADM-resistant P388 (P388/ADM) subline was established at the Taisho Research Center. Cell lines were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Grand Island Biological, Grand Island, NY, USA), 10 μM 2-hydroxyethyl disulfide (Aldrich, Milwaukee, WI, USA), and 100 IU/ml penicillin and 100 μl/ml streptomycin.

2.2. Cell survival by MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay performed in a 96-well plate was used for an in vitro chemosensitivity test (Carmichael et al., 1987). The assay is dependent on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. Equal numbers of cells (2000 for P388, P388/VCR, P388/ADM, 3000 for AD10, 1000 for K562/ADM) were inoculated into each well with 0.1 ml of culture medium. After an overnight incubation (37°C, 5% CO_2), 50 μl of vincristine or adriamycin solution, at final concentrations of 0.16–54 ng/ml for vincristine and 0.54–5400 ng/ml for adriamycin, and 50 μl of sample solution were added in triplicate and incubated as follows: 2 days for P388, P388/VCR, P388/ADM; 3 days for AD10; and 4 days for K562/ADM. Then 50 μl of MTT (1.1 mg/ml phosphate-buffered saline) were added to each well and incubated for 4 h. The resulting formazan was dissolved with 150 μl of dimethyl sulfoxide after aspiration of the culture medium. Plates were placed on a plate shaker for 5 min and read immediately at 540 nm. The median concentration 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.

2.3. Cellular uptake and retention of [^{3}H]vincristine

P388 and P388/VCR cells (2×10^6) in a tube containing 5 ml of the medium with 20 mM *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES) buffer were incubated in the presence of [^{3}H]vincristine (10 nM; specific activity 7.3 Ci/nmol) with or without modifiers (3 μg/ml), VA-033 or verapamil. At various time intervals, the cell number and the radioactivity were counted to determine the amount of cellular [^{3}H]vincristine. Upon the release of [^{3}H]vincristine, the cells were preincubated for 3 h as described above. Then, the cells were washed and further incubated with or without the modifiers.

2.4. Animals and evaluation of antitumor activity

P388/VCR cells (1×10^6/mouse) were implanted intraperitoneally into female CDF_1 mice (Japan SLC), with 7 mice in each group on day 0, and a non-effective dose (0.1 mg/kg) of vincristine (Tsuruo et al., 1981), together with VA-033 (50–200 mg/kg) and verapamil (40–60 mg/kg) were given intraperitoneally on days 1–10. Survival times were measured.

2.5. Photolabeling of [^{3}H]azidopine to the membrane vesicle of K562/ADM cells

Membrane vesicles from K562/ADM cells were prepared as described (Lever, 1977) from cells grown in 24-× 24-cm dishes under standard growth conditions (Akiyama et al., 1985). Protein concentrations were determined by the method of Bradford (1976). Membrane vesicles (50 μg of protein) were photolabeled in 40 mM Tris-HCl buffer (pH 7.2) containing 4% dimethyl sulfoxide and 200 nM [^{3}H]azidopine in a final volume of 25 μl in the presence or absence of various drugs. Photolabeled membranes were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis using gradient gels (4–20%). A total of 20 μg of protein was loaded onto each lane. The gel was fixed, treated with the fluorographic reagent Amplify (Amersham Japan), dried, and then exposed to Kodak XAR-5 films at −70°C for 10 days.

2.6. Measurement of the L-type Ca^{2+} current in the cardiac cell

Single guinea-pig ventricular cells were isolated by means of a procedure used by Kameyama et al. (1985). In brief, hearts were removed from male guinea-pigs weighing 200–300 g and mounted on a Langendorff apparatus for retrograde perfusion of the coronary circulation. After being perfused with normal Krebs-Henseleit solution, the heart was then perfused for 2–3 min with the Ca^{2+}-free Krebs-Henseleit solution, the heart was then perfused for 2–3 min with the Ca^{2+}-free Krebs-Henseleit solution equilibrated with 95% O_2/5% CO_2 at 36–37°C, followed by perfusion with the Ca^{2+}-free Krebs-Henseleit solution containing 750 U/ml collagenase (Yakult, Tokyo, Japan). The perfusion pressure was maintained at 80 mmHg. Enzymatic perfusion was stopped after 12–15 min. The heart was then washed with Kraftbrühe (KB) solution. Cells were collected and stored in KB solution at 4°C before being used in the electrophysiological experiment. The composition of solutions used in this procedure was as

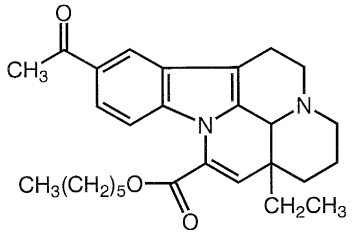

Fig. 1. Chemical structure of VA-033.

follows (mM): Krebs-Henseleit solution, 130 NaCl, 4.8 KCl, 1.2 $MgSO_4$, 1.1 NaH_2PO_4, 25 $NaHCO_3$ and 12.5 glucose; KB solution, 70 L-glutamic acid, 5 KCl, 20 taurine, 5 KH_2PO_4, 11 glucose, 5 HEPES and 0.5 EGTA free acid. Voltage-clamp experiments for the measurement of the Ca^{2+} current (I_{Ca}) were performed in the whole-cell configuration of the patch-clamp technique by using EPC-7 (List, Darmstadt, Germany) as described previously (Tsuchida et al., 1994). pCLAMP software (Axon Instruments, Foster City, CA, USA) was used for the data acquisition and analysis.

2.7. Measurement of cardiovascular functions in dogs

Beagle dogs weighing 9–12 kg were anesthetized with intravenously administered pentobarbital sodium (30

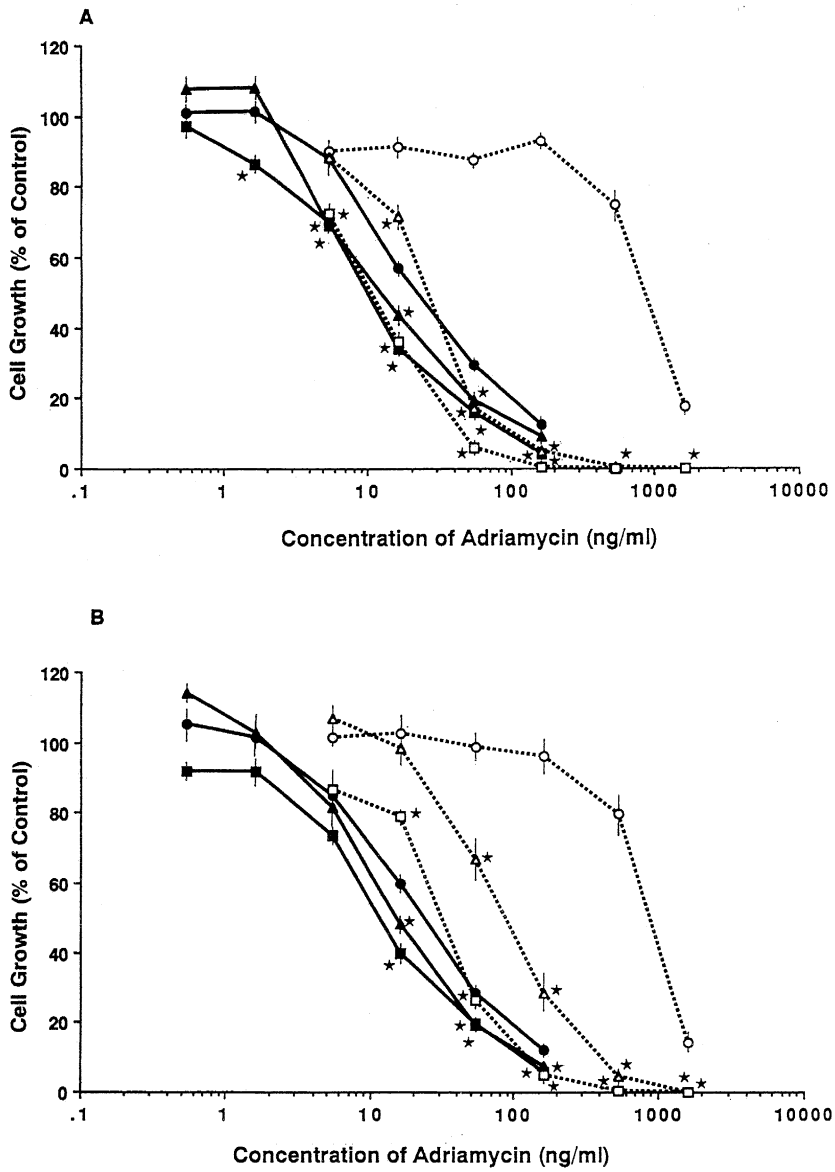

Fig. 2. Effects of VA-033 (panel A) and verapamil (panel B) on the cytotoxicity of adriamycin in P388 and P388/ADM cells. Closed circles; adriamycin only; closed triangles, adriamycin plus VA-033 or verapamil 1 μg/ml; closed squares, adriamycin plus VA-033 or verapamil 3 μg/ml, in P388 cells. Open circles, adriamycin only; open triangles, adriamycin plus VA-033 or verapamil 1 μg/ml; open squares, adriamycin plus VA-033 or verapamil 3 μg/ml, in P388/ADM cells. Values are the mean and S.E.M. of triplicate experiments. Significance of differences of values (★ $P < 0.05$) with VA-033 or verapamil from the corresponding values without VA-033 or verapamil.

mg/kg). According to the standard method, the blood pressure, heart rate, contractile force and lead II electrocardiogram were recorded. The maximum rising rate of the left ventricular pressure was taken as an index of the cardiac contractile force, and the PQ interval of the electrocardiogram was used for the determination of the atrioventricular conduction time.

2.8. Drugs

Vincristine was purchased from Shionogi (Osaka, Japan); adriamycin from Kyowa Hakko (Tokyo, Japan); [^{3}H]azidopine (50 Ci/nmol) and [^{3}H]vincristine (7.3 Ci/mmol) from Amersham Japan (Tokyo, Japan); and verapamil from Wako (Osaka, Japan). VA-033 (Fig. 1) was synthesized at the Research Center of Taisho Pharmaceutical.

2.9. Statistics

Variance of the data was analyzed with Bartlett's test, and the statistical significance of the data was determined with Dunnett's test except for survival times. Statistics for survival times were determined with the Log-Rank test. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Antitumor effects of VA-033 in vitro

VA-033 at concentrations of 1 and 3 μg/ml enhanced the cytotoxicity of adriamycin in P388 and P388/ADM cells in vitro when the cytotoxicity was judged by the cell growth inhibition caused by adriamycin in the presence or absence of VA-033. The degree of the enhancement was more marked in P388/ADM cells than in P388 cells (Fig. 2A). Verapamil at concentrations of 1 and 3 μg/ml also enhanced the cytotoxicity of adriamycin in P388 and P388/ADM cells, and its enhancing action was also stronger in P388/ADM cells than in P388 cells (Fig. 2B). The ability of VA-033 to overcome multidrug resistance in vitro was superior to that of verapamil in P388/VCR, P388/ADM and AD10 cells, and was similar to that of verapamil in K562/ADM cells at a low dose, 1 μg/ml. The ability of 3 μg/ml of VA-033 was greater than that of the same dose of verapamil in P388/ADM and AD10 cells, but smaller in K562/ADM cells (Table 1).

3.2. Effects of VA-033 on cellular uptake and retention of [^{3}H]vincristine

VA-033 at a concentration of 1 μg/ml increased the amount of cellular uptake of vincristine in both P388 and P388/VCR cells. At 5 h after incubation, the accumulation of vincristine in P388/VCR cells treated with VA-033 was 2.4 times higher than that of verapamil-treated cells (Fig. 3A). The efflux of intracellular vincristine from P388/VCR cells was inhibited by VA-033 at a concentration of 1 μg/ml. At 5 h after incubation, the retention of vincristine in P388/VCR cells treated with VA-033 was 3.1 times higher than that of the same dose of verapamil-treated cells (Fig. 3B).

3.3. Antitumor effects of VA-033 in vivo

VA-033 at intraperitoneal doses of 50 and 100 mg/kg significantly enhanced the antitumor effect of vincristine,

Table 1
Effects of VA-033 and verapamil on sensitivity of vincristine and adriamycin in multidrug-resistant cells

Modifier		Vincristine		Adriamycin	
		P388/VCR	P388/ADM	AD10	K562/ADM
Control		16.0	991	1630	872
		(1.0)	(1.0)	(1.0)	(1.0)
VA-033	0.3 μg/ml	1.03			
		(15.5)			
	1 μg/ml	0.196	24	89.7	25.6
		(81.6)	(41.3)	(18.2)	(34.1)
	3 μg/ml		10.6	32.7	24.2
			(93.5)	(49.8)	(36.0)
Control		10.2	827	769	1160
		(1.0)	(1.0)	(1.0)	(1.0)
Verapamil	0.3 μg/ml	2.4			
		(4.3)			
	1 μg/ml	0.74	93.9	92.6	36.5
		(13.8)	(8.8)	(8.3)	(31.8)
	3 μg/ml		28.2	31.7	9.25
			(29.3)	(24.3)	(125.4)

Given are IC_{50} values (ng/ml) of vincristine and adriamycin. Each value in parentheses represents relative potency of vincristine and adriamycin in the presence of VA-033 and verapamil compared with that obtained in their absence (control).

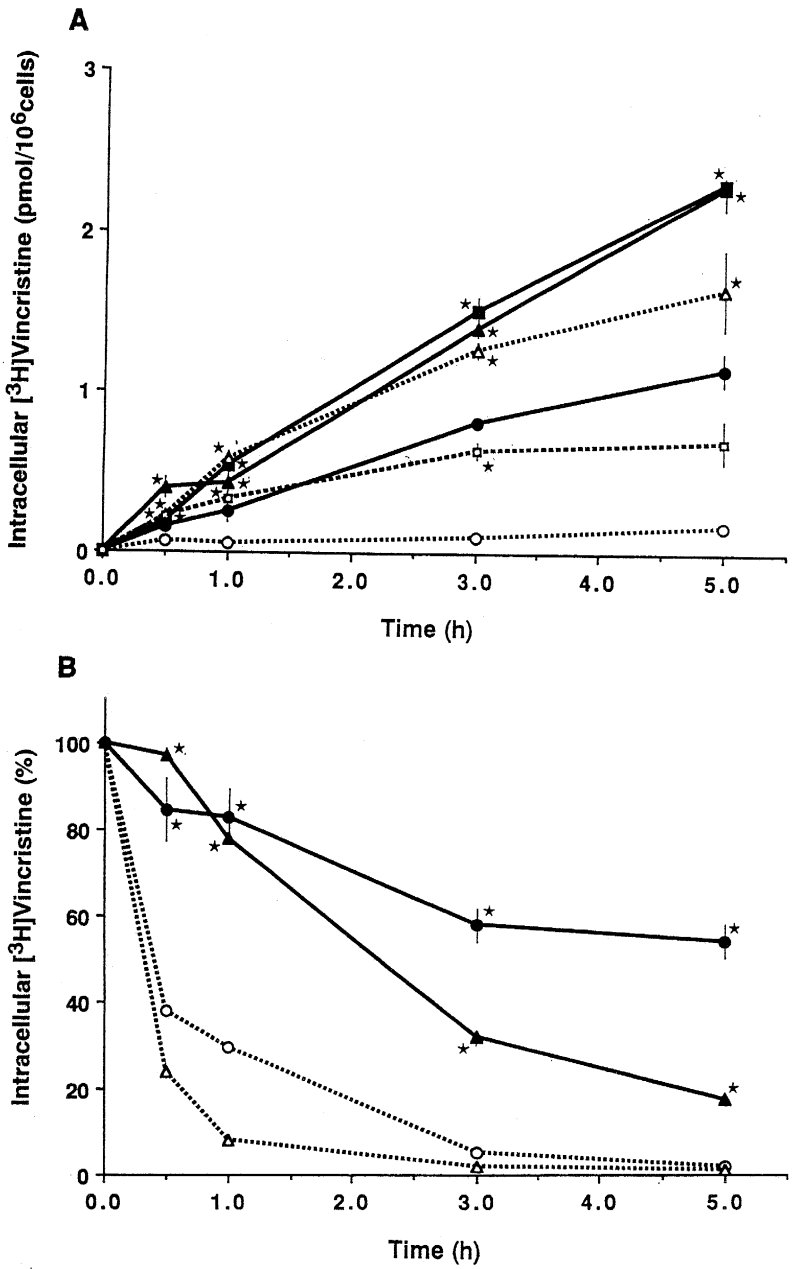

Fig. 3. Effects of VA-033 and verapamil on the uptake of [^{3}H]vincristine into P388 and P388/VCR cells (panel A), and effects of VA-033 and verapamil on the release of [^{3}H]vincristine from P388/VCR cells (panel B). Panel A: Closed circles, control; closed triangles, VA-033 1 μg/ml; closed squares, verapamil 1 μg/ml, in P388 cells. Open circles, control; open triangles, VA-033 1 μg/ml; open squares, verapamil 1 μg/ml, in P388/VCR cells. Panel B. Open circles, control; closed circles, VA-033 1 μg/ml; open triangles, control; closed triangles, verapamil 1 μg/ml, in P388/VCR cells. Values are the mean of triplicate experiments. Significance of differences of values (★ $P < 0.05$) with VA-033 or verapamil from the corresponding values without VA-033 or verapamil.

as observed by a longer survival time of the tumor cell-implanted mice treated with VA-033 compared with non-treated mice. At 200 mg/kg, the potency of the drug decreased. The dose of verapamil was determined according to our previous study (Tsuruo et al., 1981). Verapamil at doses of 40 and 50 mg/kg enhanced the antitumor effect significantly and slightly enhanced the effect at a dose of 60 mg/kg (Table 2). The highest dose of VA-033 or verapamil seemed to be somewhat toxic.

3.4. Effects of photolabeling of [^{3}H]azidopine to the membrane vesicle of K562/ADM cells

The photolabeling of P-glycoprotein in K562/ADM membranes by [^{3}H]azidopine was inhibited by VA-033 in

Table 2
Effects of VA-033 and verapamil on antitumor activity of vincristine in P388/VCR-bearing mice

Drugs	Doses	MST (days)	T/C (%)	Significance
Control		11.1	100	–
VA-033	100 mg/kg	10.8	97	NS
	200 mg/kg	11.2	101	NS
Verapamil	50 mg/kg	10.2	92	NS
	60 mg/kg	10.3	93	NS
Vincristine	0.1 mg/kg	13.1	118	NS
+VA-033	50 mg/kg	15.8	142	$P < 0.05$
	100 mg/kg	16.3	147	$P < 0.05$
	200 mg/kg	12.5	113	NS
+verapamil	40 mg/kg	15.0	135	$P < 0.05$
	50 mg/kg	16.1	145	$P < 0.05$
	60 mg/kg	16.0	144	NS

MST, Median survival time; T/C, the ratio of median survival time in the drug-treated group versus that in the control group. Significance was determined versus control. NS, not significant.

a concentration-dependent manner, and VA-033 at 10 μM partially, and at 100 μM completely inhibited the P-glycoprotein labeling. Verapamil at 100 μM also partially inhibited the P-glycoprotein labeling, but at 10 μM had no inhibition at all (Fig. 4).

3.5. Ca^{2+} antagonistic effects of VA-033

To monitor the I_{Ca}, ventricular myocytes were depolarized by a test pulse with a duration of 300 ms from −40 mV and at increasing voltage steps of 10 mV every 5 s. The amplitude of the I_{Ca} was established by measuring the difference between the value of the peak inward currents and that of the currents at the end of 300-ms test pulses. VA-033 at concentrations of less than 100 μM had no

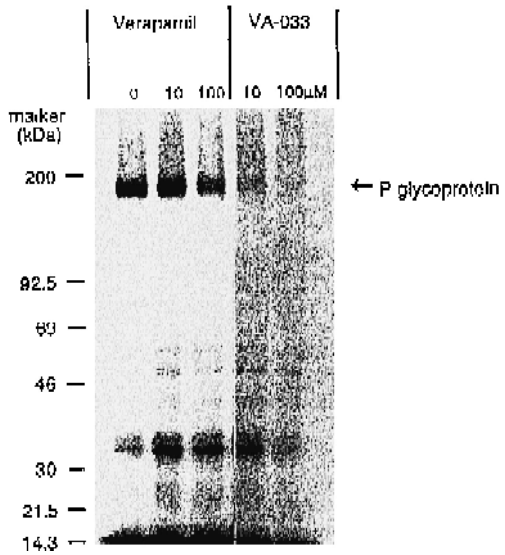

Fig. 4. The inhibitory effects of VA-033 and verapamil on [^{3}H]azidopine photolabeling to P-glycoprotein. Typical photolabeling patterns are presented. Another experiment showed the same pattern.

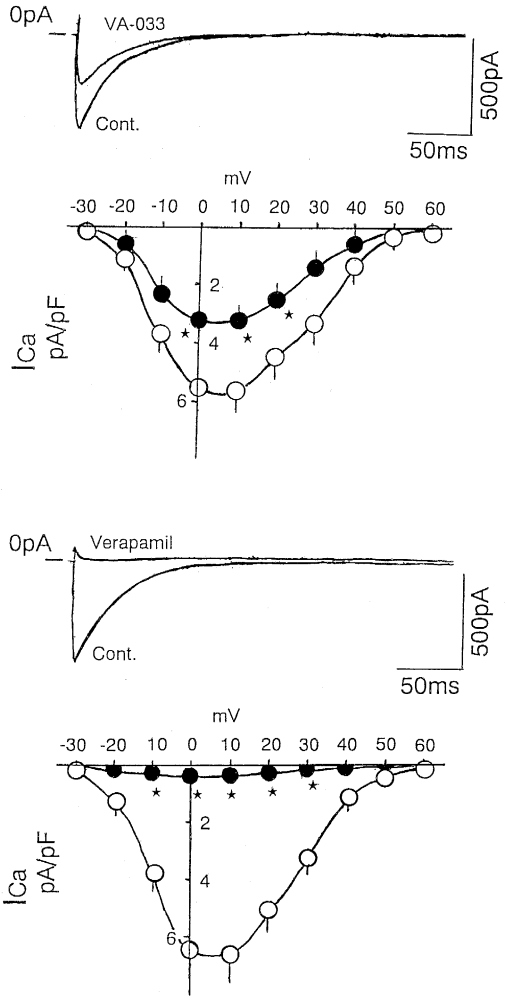

Fig. 5. The inhibitory effects of VA-033 and verapamil on the Ca^{2+} current (I_{Ca}) in single guinea-pig ventricular myocytes. Typical tracings of I_{Ca} induced by a test pulse to 10 mV (in insets) and current–voltage relationships in the presence of VA-033 300 μM or verapamil 3 μM, or in its absence (control). In the upper panel, open circles represent control and closed circles represent VA-033 300 μM. In the lower panel, open circles represent control and closed circles represent verapamil 3 μM. Each $n = 4$. Bars represent S.E.M. * $P < 0.05$ versus control values.

significant effects on the I_{Ca}. At 300 μM, it inhibited the I_{Ca} by 41% at a test pulse of 10 mV, whereas verapamil at a concentration of 3 μM inhibited the I_{Ca} by 95% at a test pulse of 10 mV (Fig. 5).

3.6. Effects of VA-033 on the cardiovascular functions in dogs

VA-033 was resolved in dimethyl sulfoxide at a concentration of 100 mg/ml. The solvent used for VA-033 resolution slightly affected cardiovascular parameters, as shown in Fig. 6. VA-033 at intravenous doses of 0.1–1 mg/kg did not affect cardiovascular parameters. VA-033 at 3 mg/kg significantly decreased only diastolic blood pressure compared with the equivalent dose of solvent

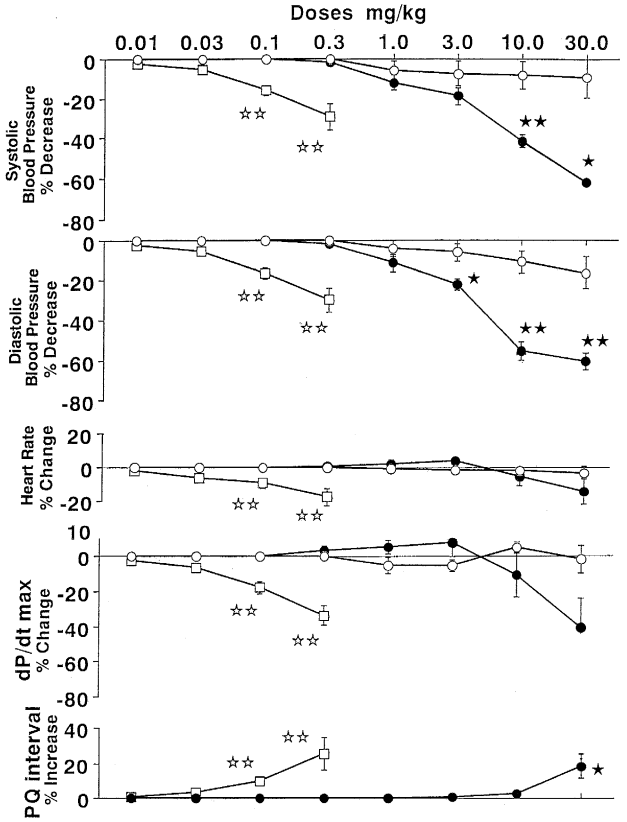

Fig. 6. The dose–response relationships of the cardiovascular effects of VA-033 and verapamil in anesthetized dogs. Cardiovascular parameters represented are as follows: systolic blood pressure, diastolic blood pressure, heart rate, maximum rising rate of left ventricular pressure (dP/dt_{max}) and PQ interval of the electrocardiogram (PQ interval). Closed circles and open circles represent VA-033 and solvent for VA-033 (each $n = 4$), and open squares represent verapamil ($n = 6$). Bars indicate S.E.M. ☆☆ $P < 0.01$ versus the pre-values (the base-line values before verapamil administration), ★ $P < 0.05$, ★★ $P < 0.01$ versus the values after the equivalent amount of the solvent resoluted for the corresponding VA-033.

VA-033 at 10 and 30 mg/kg which decreased systolic and diastolic blood pressure significantly. However, other parameters such as heart rate and cardiac contractile force (maximum rising rate of the left ventricular pressure; LV dP/dt_{max}) were not affected significantly with VA-033 at dose ranges of 0.1–30 mg/kg. The PQ interval of the electrocardiogram was significantly prolonged with 30 mg/kg of VA-033. On the other hand, although verapamil at 0.01 and 0.03 mg/kg did not affect cardiovascular parameters significantly, verapamil at 0.1 and 0.3 mg/kg decreased systemic blood pressure, heart rate and LV dP/dt_{max}, and prolonged the PQ interval significantly (Fig. 6). The solvent for verapamil had no effect on these parameters ($n = 2$; data not shown). Atrioventricular block was observed in none of 4 dogs with even the highest dose (30 mg/kg) of VA-033, while it was observed in 2 of 6 dogs with 0.3 mg/kg of verapamil. The potency of the decreasing effects of VA-033 on systemic blood pressure was 3 or more times lower than that of verapamil, and VA-033 was about 10–100 times less effective than verapamil in depressing the cardiac function as shown by decreased LV dP/dt_{max} and increased PQ interval (Fig. 6). The half-duration of the decrease in mean blood pressure was 2.2 ± 0.3 and 5.4 ± 0.8 min for 10 and 30 mg/kg of VA-033, respectively, and 2.6 ± 0.2 and 4.7 ± 0.3 min for 0.1 and 0.3 mg/kg of verapamil, respectively (mean ± S.E.M.). It seems that the cardiac action of VA-033 is relatively weaker than its vascular actions compared with the profile of actions of verapamil in terms of heart or vessels (Fig. 6).

4. Discussion

Drug resistance is a crucial problem in the treatment of cancer. Apart from intrinsically resistant tumors, some sensitive tumors often gradually develop drug resistance during multiple cycles of chemotherapy. A variety of mechanisms of drug resistance are considered, including reduced cellular drug accumulation, increased detoxification, intracellular vesicularization of drugs, altered enzymatic activities, down-regulation of target and enhanced DNA repair (Beck, 1990). The expression of P-glycoprotein in the membranes of tumor cells leads to cross-resistance to chemically unrelated cytostatic drug, because P-glycoprotein has been demonstrated to have more than two distinct drug acceptor sites that allosterically couple (Ferry et al., 1992; Borchers et al., 1995). P-glycoprotein is an ATP-dependent drug efflux pump that transports different cytostatic drugs out of the neoplastic cells, resulting in low and ineffective intracellular drug concentration, and in multidrug resistance. It is well known that neoplastic cells resistant to multiple drugs arise with doxorubicin, vincristine, vinblastine, and actinomycin D after cell exposure to these antineoplastic agents (Biedler and Riehm, 1970). It has been demonstrated that verapamil, dihydropyridine derivatives, quinidine, reserpine, and other drugs reverse the multidrug resistance (Inaba et al., 1981; Tsuruo et al., 1981, 1984; Niwa et al., 1992). These drugs are supposed to inhibit the activity of P-glycoprotein to pump out chemotherapeutic agents from the cells through binding with P-glycoprotein. (Cornwell et al., 1987; Akiyama et al., 1988; Kamiwatari et al., 1989). In this study, we examined the reversing effects of a newly synthesized apovincaminic acid ester, VA-033, on multidrug resistance because it has been demonstrated that vinca alkaloids such as vincristine are known to bind P-glycoprotein, and agents capable of binding P-glycoprotein often show chemotherapeutic effects on neoplastic tissues or reverse the multidrug resistance. It was found that VA-033 exerted the reversing effects on multidrug resistance in vitro and in vivo. The degree of the reversing potency was stronger than that of verapamil in vitro. Photoaffinity labeling to P-glycoprotein is a valuable technique for evaluation of drug binding sites

and the elucidation of the mechanism of multidrug resistance modifiers. An often used photoaffinity labeling agent for P-glycoprotein has been [^{3}H]azidopine (Safa et al., 1987). VA-033 inhibited the azidopine photolabeling more strongly compared with verapamil. Furthermore, VA-033 augmented the intracellular accumulation of vincristine in the neoplastic cells. Thus, it is considered that VA-033 increased the accumulation of chemotherapeutic agents in the neoplasmic cells by inhibiting the pump activity of P-glycoprotein. In contrast to the in vitro study, the enhancing potency of VA-033 on the antitumor activity of vincristine in P388/VCR-bearing mice was not necessarily stronger than that of verapamil. We have not yet conducted detailed experiments examining the exact bioavailability of VA-033, so a possible lower bioavailability due to higher metabolism and/or excretion compared with verapamil may be one of the causes for the limited in vivo potency of VA-033 in mice. However, VA-033 showed a smaller decrease in blood pressure, cardiac contractility, and a smaller atrio-ventricular conduction delay than verapamil. Atrio-ventricular conduction block was not observed with VA-033, differing from verapamil. This is considered to be due to the weaker Ca^{2+} antagonistic effect induced by VA-033 compared with verapamil, as shown in the slight inhibitory effect on I_{Ca} in ventricular myocytes. VA-033 is expected to be more beneficial in clinical trials because of weaker side effects on the cardiovascular functions compared with verapamil.

In conclusion, as a new multidrug resistance modifier, VA-033 showed strong resistance-reversing activity by a mechanism similar to that of verapamil; VA-033 increases intracellular concentrations of chemotherapeutic agents by inhibiting the function of P-glycoprotein. Furthermore, the effects of VA-033 on cardiovascular functions were less than those of verapamil. These properties make VA-033 a candidate of multidrug resistance modifiers for further investigation.

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