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Potentiation of Some Anticancer Agents by Dipyridamole against Drug-sensitive and Drug-resistant Cancer Cell Lines

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In this study, we have used two different vincristine (VCR)-resistant variants, VJ-300 and HC-7-5/VCR. VJ-300 was isolated from a human cancer KB cell line and HC-7-5/VCR from a human cancer HC-7-5 cell line. VJ-300 and HC-7-5/VCR are both multidrug-resistant (MDR) variants, showing resistance to multiple anticancer drugs such as VCR, adriamycin, actinomycin D and daunomycin. Dipyridamole, a specific inhibitor of nucleoside transport, potentiated these anticancer drugs about 2- to 10-fold against KB and VJ-300. Dipyridamole almost completely reversed drug resistance to actinomycin D in VJ-300 cells with about a 70-fold higher resistance to actinomycin D. Dipyridamole inhibited the efflux of actinomycin D and VCR from VJ-300 cells. Dipyridamole enhanced the uptake of VCR but not that of actinomycin D in VJ-300 and KB. Dipyridamole at 10–100 μM inhibited photoaffinity labeling with [^{3}H]azidopine of the cell-surface protein P-glycoprotein in VJ-300 cells. Dipyridamole potentiated 5-fluorouracil and hexylcarbamoyl-5-fluorouracil in cultured KB and VJ-300, but it annihilated the cytotoxic action of 5-fluorouridine. Potentiation of 5-fluorouracil by dipyridamole against HC-7-5 and HC-7-5/VCR was also observed, but appeared to be less than in VJ-300 and KB cells. Dipyridamole almost completely inhibited the cellular accumulation of 5-fluorouridine, but not that of 5-fluorouracil. Thus, dipyridamole appeared to potentiate anticancer agents through pleiotropic action sites, one of which is inhibition of enhanced efflux of MDR cell lines and the other of which is inhibition of nucleoside transport. Dipyridamole might be a useful and potent agent to potentiate anticancer agents and reverse drug-resistance.

Key words: Drug resistance — Potentiation — Combination therapy — Dipyridamole

Dipyridamole (DPM), a potent inhibitor of membrane nucleoside transport,[1-3] has been used clinically as a vasodilator and antiplatelet agent. DPM has recently been reported to potentiate various anticancer agents such as methotrexate, 5-fluorouracil (5-FU) and vincristine (VCR),[4-11] and some of these combinations have been used clinically against malignant tumors.[12] In contrast, combinations of anticancer agents with verapamil or cepharanthine have been found to reverse multidrug-resistance (MDR), possibly through inhibition of drug efflux from tumor cells,[13-16] and photoaffinity labeling of P-glycoprotein by N-(*p*-azido[3-^{125}I]salicyl)-N′-β-aminoethylvindesine (^{125}I-NASV).[16-18] Both verapamil and cepharanthine have long been used clinically for different purposes from their MDR-reversal actions. In this study, we examined whether or not DPM could potentiate anticancer agents and whether DPM could overcome MDR in MDR variants of cultured drug-sensitive human cancer cell lines.

MATERIALS AND METHODS

Cell line and cell culture We used two MDR cell lines, VJ-300 derived from a human cancer KB cell line,[19] and HC-7-5/VCR derived from a human cancer HC-7-5 cell line.[20] Both VJ-300 and HC-7-5/VCR were isolated after stepwise exposure to VCR. VJ-300 and HC-7-5/VCR showed 260-fold and 100-fold higher resistance to VCR, respectively, than their parental cell lines. VCR-resistance in VJ-300 has been maintained in a stable state during culture in the absence of VCR for more than six months.[19] In contrast, VCR-resistance in HC-7-5/VCR has been found to revert almost completely after incubation for 3 months in the absence of VCR.[20] HC-7-5/VCR has been routinely maintained by culturing in the presence of 300 ng/ml VCR,[20] but VJ-300 has been maintained in the absence of any agent. KB cells were grown in monolayer in MEM (Nissui Seiyaku Co., Tokyo) containing 10% newborn calf serum (Microbiological Associates, Bethesda, MD), 1 mg/ml Bactopeptone (Difco Laboratories, Detroit, MI), 0.292 mg/ml glutamine, 100 μg/ml kanamycin, and 100 units/ml penicillin as described previously.[21]

Drugs and chemicals DPM was supplied by Nippon Boehringer Ingelheim Co., Ltd, Kawanishi. 5-FU, 5-fluorouridine (5-FUR), daunomycin (DAU), adriamycin (ADM), VCR and actinomycin D (ACD) were obtained from Sigma Chemical Co., St. Louis, MO. Cepharanthine was obtained from Kaken Shoyaku Co., Tokyo. Hexylcarbamoyl-5-fluorouracil (HCFU) was ob-

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tained from Mitsui Pharmaceuticals Inc., Tokyo. [^{3}H]-VCR (4.8 Ci/mmol) and 5-fluoro-6-[^{3}H]uridine ([^{3}H]5-FUR) (16 Ci/mmol) were purchased from New England Nuclear and [^{3}H]ACD (3.3 Ci/mmol), [^{3}H]azidopine (40 Ci/mmol) and 5-fluoro-6-[^{3}H]uracil ([^{3}H]5-FU) (2.5 Ci/mmol) were purchased from Amersham.

Cell survival assay by colony formation Cell survival was determined by colony formation assay as described previously.[15, 21] In brief, 400 cells were plated in 60 mm dishes in the absence of any drug. Various drugs were added 18 h later, and the colonies which appeared were scored using Giemsa staining after incubation for 10 days at 37°C. Solutions of all drugs were freshly prepared before use in dimethyl sulfoxide. The relative resistances were determined by dividing the D_{10} of the VJ-300 (or HC-7-5/VCR) cells with or without DPM by the D_{10} of the KB (or HC-7-5) cells without DMP; D_{10} was the concentration of drug which reduced the surviving fraction to 10% of the control (without drugs). The amount of DPM used allowed 90% to 100% of the KB, VJ-300, HC-7-5 and HC-7-5/VCR cells to survive.

Drug accumulation Cells (4×10^5) were plated in a 35-mm plastic dish and incubated for 48 h at 37°C. The medium was replaced with serum-free medium, and the cells were subsequently incubated with either 0.25 μCi/ml [^{3}H]VCR or [^{3}H]ACD in the presence or absence of DPM for 2 h. The cells were also incubated with either 0.1 μCi/ml [^{3}H]5-FU or [^{3}H]5-FUR for 2 h in order to assay the accumulation of 5-FU and 5-FUR. The cells were washed once with cold phosphate-buffered saline (PBS) (g/liter; NaCl, 8.0; Na_2HPO_4 $12H_2O$, 2.9; KCl, 0.2; KH_2PO_4, 0.2) then harvested. The cells were washed 3 times with cold PBS, and the cellular pellets were suspended in 0.7 ml of H_2O and mixed thoroughly with 7 ml of Scintisol EX-H (Wako Chemical Co., Osaka), then their radioactivity was determined.[15, 16, 21]

Drug efflux and influx In order to study the drug efflux, exponentially growing KB and VJ-300 cells (4×10^5) were plated in 35-mm dishes and incubated for 48 h at 37°C, and then for 1 h at 37°C with DPM in a fresh serum-free medium. The KB and VJ-300 cells were subsequently incubated with 0.20 μCi/ml and 2 μCi/ml [^{3}H]-VCR or with 0.20 μCi/ml and 2 μCi/ml [^{3}H]ACD, respectively, at 37°C for 120 min to obtain a similar accumulation of both radioactive compounds. Each dish was washed 3 times with PBS and fresh serum-free medium was added either with or without DPM. Incubation was continued for the indicated times at 37°C, then the cells were harvested, and the intracellular redioactivity was counted.[15, 16, 21] In order to study the drug influx, cells were plated and incubated for 48 h, and the medium was changed to a glucose-free, serum-free Hanks' balanced salt solution. The cells were pre-incubated for 1 h at 37°C either with or without DPM, and then exposed to 0.5 μCi/ml [^{3}H]VCR or [^{3}H]ACD for 1 min. Each dish was washed 3 times with ice-cold PBS, cells were harvested, and then the intracellular radioactivity was counted.[15, 16, 21]

Photoaffinity labeling Membrane vesicles were incubated with [^{3}H]azidopine (1.5 μCi/assay) for 15 min at room temperature with or without DPM or cepharanthine.[17, 22, 23] After irradiation for 20 min at 25°C, the samples were solubilized in sodium dodecyl sulfate (SDS) buffer as described previously.[23] The samples labeled with [^{3}H]azidopine were fractionated by electrophoresis on SDS-polyacrylamide-urea gel using a modification of the system described by Debenham *et al.*[24] The gel bed consisted of 5% polyacrylamide/4.5 *M* urea gel, pH 7.6, without a stacking gel.[16]

RESULTS

Potentiation by DPM of VCR and ACD An MDR-cell line, VJ-300, derived from human cancer KB cell line was used to determine whether DPM could overcome MDR. An example of our colony formation assays to test the effect of combinations of DPM and VCR, and DPM and ACD is shown in Fig. 1. VJ-300 showed 260-fold higher resistance to VCR than KB. Combinations of VCR with either 10 or 20 μM DPM showed potentiation of VCR on both KB and VJ-300. DPM augmented the cytocidal action of VCR about 5-fold on KB and about

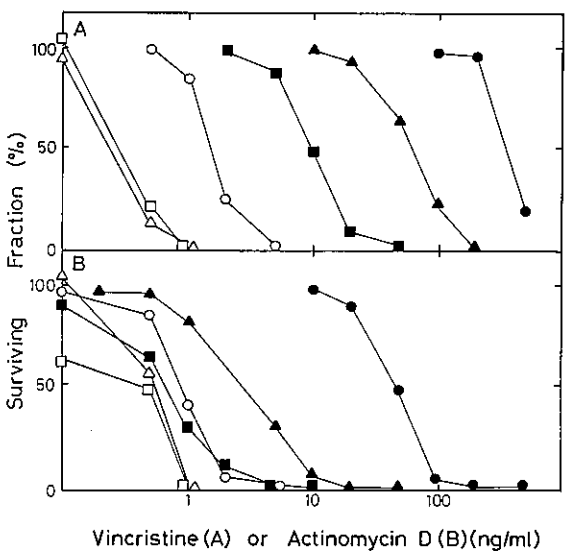

Fig. 1. Effect of DPM on drug-resistance in KB cells. The effect of DPM on resistance to VCR (A) and ACD (B) in KB (○, △, □) and VJ-300 (●, ▲, ■) cells in the presence of 0 μM (○, ●), 10 μM (△, ▲) and 20 μM (□, ■) DPM was examined by colony formation assay. Each point is the average of duplicate dishes.

Table I. Effect of DPM on Cellular Sensitivities to VCR, ADM, DAU and ACD of KB and VJ-300 Cells

Cell lines	DPM (μM)	Relative resistance[a]			
		VCR	ADM	DAU	ACD
KB	0	1.0	1.0	1.0	1.0
	10	0.3	0.4	0.9	0.8
	20	0.2	0.4	0.5	0.5
VJ-300	0	260.0	14.0	11.0	71.0
	10	44.0	10.0	9.0	5.0
	20	23.0	6.0	5.0	1.0

a) The values for relative resistance to various anticancer agents are the means of triplicate experiments.

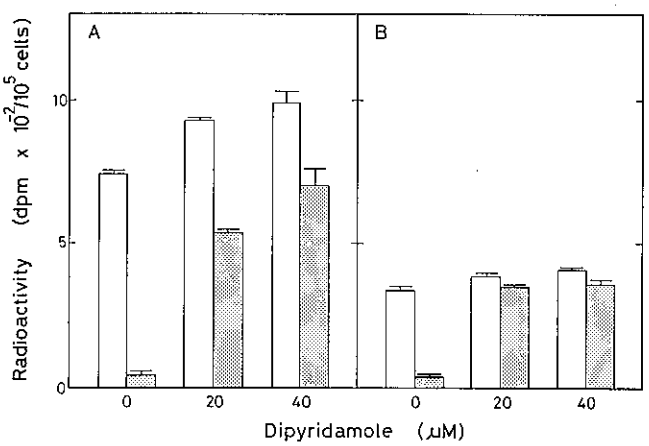

Fig. 2. Effect of DPM on accumulation of [^{3}H]VCR and [^{3}H]ACD in KB and VJ-300 cells. The intracellular levels of VCR (A) and ACD (B) in the presence of DPM in KB (□) and VJ-300 (▨) cells were determined as described in "Materials and Methods." Columns, the means of triplicate experiments; bars, SD.

20-fold on VJ-300 cells (Fig. 1A). As can be seen in Fig. 1B, VJ-300 was resistant to a 70-fold higher dose of ACD than KB, and a combination of ACD with 20 μM DPM almost completely overcame ACD-resistance in the VJ-300 cells, resulting in a level of ACD-sensitivity similar to that in KB cells. DPM had much less effect on ACD-sensitivity in KB cells; it increased the cytotoxic action of ACD against KB cells by about 2-fold.

Table I summarizes the data from dose-response curves of various combinations of DPM and four anticancer agents (VCR, ADM, DAU and ACD). In VJ-300 cells, ACD-resistance was almost completely overcome in combination with DPM, but VCR-resistance was only partially overcome. DPM showed about a 2-fold increase in the cytotoxic activity of ADM and DAU against VJ-300 cells. DPM at 10 or 20 μM increased the cytocidal

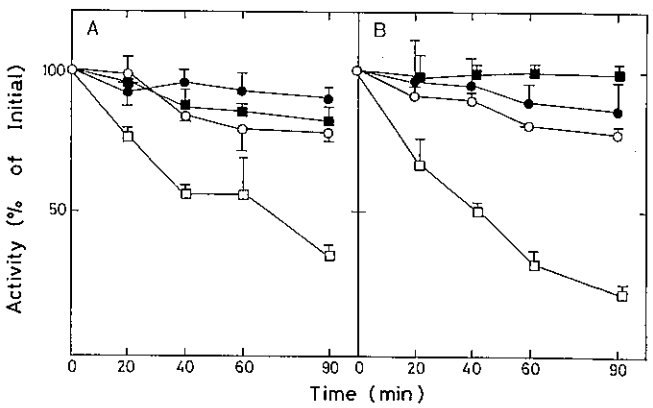

Fig. 3. Effect of DPM on efflux of [^{3}H]VCR and [^{3}H]ACD. Efflux of VCR (A) and ACD (B) in the absence (○, □) or presence (●, ■) of 40 μM DPM from KB (○, ●) and VJ-300 (□, ■) cells is shown. Points, the means of triplicate experiments; bars, SD.

action of ADM, DAU and ACD only 2-fold against KB cells while DPM increased the cytotoxic activity of VCR on KB cells 5-fold.

Effect of DPM on cellular accumulation, efflux and influx The intracellular level of VCR in VJ-300 cells in the absence of DPM was about one-twentieth of that of KB cells (Fig. 2A). The accumulation of VCR in KB cells treated with DPM increased by as much as 1.4-fold. In contrast, the accumulation of VCR in VJ-300 cells with 40 μM DPM increased about 18-fold, but the amount of accumulation of VJ-300 cells with DPM was still less than that of KB cells without DPM. Figure 2B shows that the intracellular level of ACD in VJ-300 cells in the absence of DPM is about one-ninth of that of KB cells. In the presence of DPM the accumulation of ACD in VJ-300 cells increased about 10-fold and it was as much as that of KB cells without DPM. In comparison with VJ-300 cells, the accumulation of either ACD or VCR with DPM was less than 40% of the increase of either accumulation in KB cells in the absence of DPM.

To test the effect of DPM on drug efflux activity, the cells were exposed to [^{3}H]VCR or [^{3}H]ACD for 2 h followed by incubation either with or without DPM at 40 μM in MEM containing no serum (Fig. 3). After incubation of the cells for 90 min in the absence of DPM, more than 60% of the VCR was lost from the VJ-300 cells, whereas about 80% of the VCR was retained in the KB cells (Fig. 3A). Addition of 40 μM DPM to the culture medium almost completely inhibited the efflux of VCR from the VJ-300 cells and slightly decreased the efflux from the KB cells. The effect of DPM on the efflux of ACD in both KB and VJ-300 cells was very similar to that of VCR (Fig. 3B). DPM specifically inhibited the

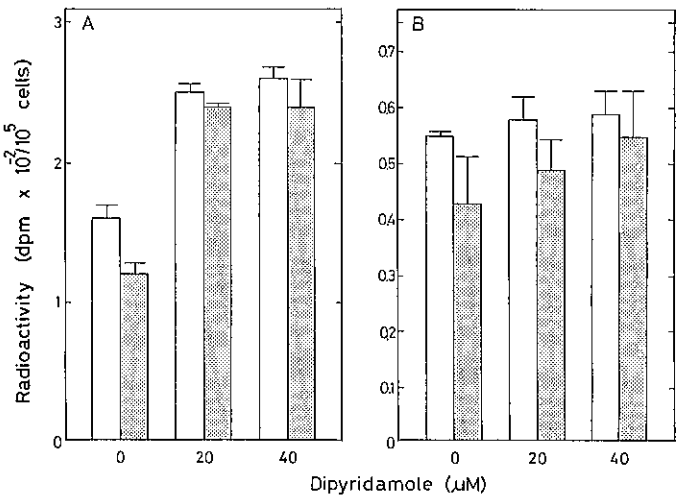

Fig. 4. Effect of DPM on influx of [^{3}H]VCR and [^{3}H]ACD. Influx of VCR (A) and ACD (B) in the absence or presence of 20 or 40 μM DPM: KB (□) and VJ-300 (▨). Columns, the means from triplicate experiments; bars, SD.

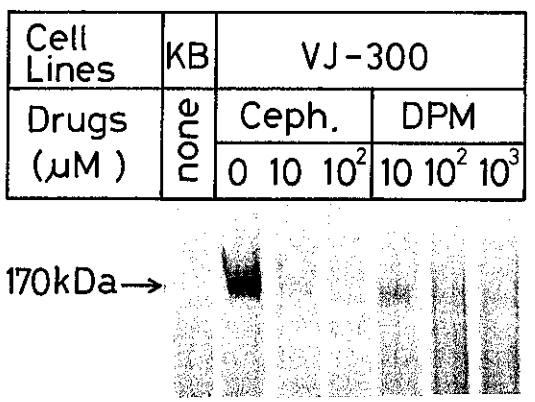

Fig. 5. The inhibition of [^{3}H]azidopine labeling of the 170 kDa P-glycoprotein in membrane vesicles by DPM. VJ-300 vesicles (80 μg of protein per lane) were incubated with [^{3}H]-azidopine in the absence or presence of the indicated concentration of cepharanthine (Ceph.) or DPM. Autoradiograms were developed after one week of exposure. The arrow indicates the expected position of a 170 kDa band based on the use of molecular size markers. KB vesicles (80 μg of protein per lane) were also examined as a control.

efflux of ACD from VJ-300 cells. The effect of DPM on the influx of VCR and ACD was also examined (Fig. 4). The influx of VCR was apparently increased with DPM in KB (1.6-fold) and VJ-300 (2-fold) (Fig. 4A). On the other hand, the influx of ACD increased only slightly, if at all, with DPM in both KB and VJ-300 cells (Fig. 4B).

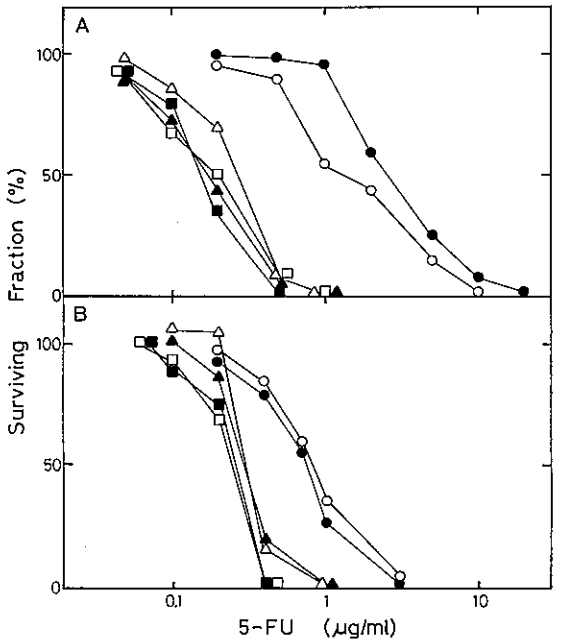

Fig. 6. Effect of DPM on the cytocidal action of 5-FU on KB and VJ-300 cells (A), and on HC-7-5 and HC-7-5/VCR cells (B). Dose-response curves of the four cell lines to 5-FU in the absence or presence of DPM were obtained by assay of colony-forming ability. In (A), KB (○, △, □) and VJ-300 (●, ▲, ■) were exposed to 0 μM (○, ●), 10 μM (△, ▲) and 20 μM (□, ■) DPM. In (B), HC-7-5 (○, △, □) and HC-7-5/VCR (●, ▲, ■) were exposed to 0 μM (○, ●), 5 μM (△, ▲) and 10 μM (□, ■) DPM. Each point is the mean of duplicate dishes.

Photoaffinity labeling of P-glycoprotein by azidopine [^{3}H]Azidopine specifically labels the P-glycoprotein of 170,000 molecular weight which is overexpressed in membrane vesicles of multidrug-resistant cells.[23, 25] Agents which reverse multidrug-resistance, such as verapamil and other calcium channel blockers, interfere with this photoaffinity labeling.[23, 25] We thus examined whether DPM inhibited the labeling of P-glycoprotein in membrane vesicles of VJ-300 cells by [^{3}H]azidopine. As can be seen in Fig. 5, cepharanthine at 10 μM almost completely inhibited photoaffinity labeling. DPM also inhibited the photoaffinity labeling by [^{3}H]azidopine at 10–100 μM, suggesting interaction of DPM with P-glycoprotein.

Potentiation of 5-FU by DPM DPM specifically inhibits uridine transport in cultured mammalian cells through interaction with nucleoside transporters.[26] The cytotoxic effect of 5-FU against human colon cancer cells is augmented by DPM.[8] We thus determined whether DPM could potentiate 5-FU against two MDR cell lines, VJ-300 and HC-7-5/VCR. Figure 6 shows the dose-response

curves of KB and VJ-300 (Fig. 6A) and those of HC-7-5 and HC-7-5/VCR (Fig. 6B) to 5-FU when combined with or without DPM. The combination of 5-FU with either 10 or 20 μM DPM showed about a 10-fold potentiation by DPM of 5-FU action against both KB and VJ-300 cells. In contrast, 5 or 10 μM DPM gave about a 2- to 3-fold potentiation of 5-FU against HC-7-5 and HC-7-5/VCR. Cell survival was not affected by DPM alone at 5 μM for KB or VJ-300 or by DPM alone at 10 μM for HC-7-5 or HC-7-5/VCR. DPM alone inhibited slightly less than 10% of the initial surviving fraction at 10 μM for KB and VJ-300 and at 20 μM for HC-7-5 and HC-7-5/VCR.

As can be seen in Table II, VJ-300 and HC-7-5/VCR showed 70- and 80-fold higher resistance to ACD, respectively, than their parental cell lines. Table II summarizes data from dose-response curves of various combinations of DPM and anticancer agents. Although DPM increased the cellular sensitivity to ACD in both of the drug-sensitive KB and HC-7-5 cell lines by only 2-fold, it almost completely overcame ACD-resistance in their MDR cell lines, VJ-300 (see also Fig. 1 and Table I) and HC-7-5/VCR. Table II also shows a 10-fold or more potentiation of 5-FU and HCFU, a synthetic antitumor analog of 5-FU,[27] by DPM against both KB and VJ-300 cells. DPM however shows only a 2-fold potentiation of 5-FU against HC-7-5 and HC-7-5/VCR cells. By contrast, DPM virtually annihilated the cytotoxic effects of 5-FUR against all four cell lines, KB, VJ-300, HC-7-5 and HC-7-5/VCR.

Table II. Effect of DPM on Cellular Sensitivities to 5-FU, 5-FUR, HCFU and ACD[a]

Cell lines	DPM (μM)	Relative resistance			
		ACD	5-FU	HCFU	5-FUR
KB	0	1.0	1.0	1.0	1.0
	10	0.4	0.07	0.06	10.0
VJ-300	0	70.0	1.2	1.1	1.8
	10	0.9	0.06	0.08	17.0
HC-7-5	0	1.0	1.0	—[b]	1.0
	5	0.5	0.4	—	58.0
HC-7-5/VCR	0	80.0	1.4	—	1.2
	5	2.0	0.6	—	60.0

a) The values for relative resistance to various anticancer agents are the means of duplicate experiments.
b) Not tested.

Table III. Cellular Accumulation of 5-FU and 5-FUR in KB and VJ-300 Cells in the Presence of DPM[a]

Radioactive compound	DPM (μM)	Radioactivity(dpm/10^5 cells)	
		KB	VJ-300
[^{3}H]5-FU	0	2,505	2,708
	40	2,475	2,698
[^{3}H]5-FUR	0	28,900	27,800
	40	250	325

a) $3–5\times10^5$ KB and VJ-300 cells were exposed to [^{3}H]5-FU or [^{3}H]5-FUR for 2 h at 37°C in the absence and presence of DPM, and cell-associated radioactivity was measured. The values are the means of duplicate trials. The duplicate determinations varied by less than 10% of the average.

DPM specifically inhibits cellular transport of nucleoside compounds such as uridine or thymidine.[26] The effect of DPM on the cellular accumulation of 5-FU and 5-FUR was compared between KB and its MDR-cell line, VJ-300 (Table III). The intracellular levels of [^{3}H]-5-FU and [^{3}H]5-FUR were similar in both KB and VJ-300 cells in the absence of DPM. Treatment with 40 μM DPM did not significantly affect the accumulation of 5-FU in KB or VJ-300 whereas DPM decreased cellular accumulation of 5-FUR to about 1/100 of the controls in both cell lines (Table III). Time courses of the cellular uptake of 5-FU and 5-FUR showed a linear increase as a function of incubation time up to 12 h (data not shown). In the KB and VJ-300 cell lines, DPM also appeared to inhibit the cellular transport of a nucleoside analog (5-FUR), but not that of a base analog (5-FU). We also observed the complete inhibition of transport of [^{3}H]-uridine, but not of [^{3}H]uracil (data not shown). These data suggest the existence of a DPM-sensitive nucleoside transport system in KB and its MDR variant.

DISCUSSION

Verapamil,[13,28] phenothiazine calmodulin inhibitors,[14] synthetic isoprenoids,[21,29] retinyl acetate[22] and cepharanthine[15] rather specifically overcome multidrug-resistance to DAU, ACD, ADM and VCR in cultured MDR cell lines, but they do not potentiate many anticancer agents against drug-sensitive cell lines. Some synthetic isoprenoids, however, have been shown to potentiate bleomycin and other anticancer agents.[30] In contrast, DPM was found to show a unique property. DPM can partially reverse drug-resistance to VCR, DAU and ADM, and it can almost completely reverse ACD-resistance in an MDR cell line of KB, VJ-300. DPM also augments the cytocidal actions of VCR, DAU, ADM and ACD against drug-sensitive KB cells, and DPM can potentiate 5-FU or its analogue, HCFU, against both drug-sensitive and drug-resistant cell lines. Our independent *in vitro* assays show that DPM at 10 μM can potentiate VCR, 5-FU, etoposide (VP-16), teniposide (VM-26), methotrexate and melphalan against human prostate cancer PC-3 cells, but it cannot potentiate nitrosourea, *cis*-platinum or carboplatinum (Y. Saburi, un-

published data). These data suggest that DPM has unique properties among MDR-reversal agents.

Most agents that overcome multidrug resistance enhance the cellular accumulation of VCR, DAU or ACD in MDR cells possibly through inhibition of drug efflux,[13-16, 21, 31] and they specifically bind to a plasma membrane glycoprotein of 170,000 molecular weight which appears to be an efflux pump for certain anticancer agents.[16, 17, 23, 32] Hirose *et al.*[10] have shown synergistic effects of DPM and VCR or vindesine, possibly due to an enhanced accumulation of these anticancer agents by DPM in cultured human leukemic and lymphoma cell lines. Kusumoto *et al.*[11] have also shown synergistic effects of DPM and ADM against HeLa cells in culture and against growth of sarcoma 180 cells *in vivo*. In DPM-treated HeLa cells cellular accumulation of VCR is increased about 1.5-fold, possibly due to an enhanced uptake rather than blockage of drug efflux. Our present study shows an inhibition of efflux activity for VCR and ACD, resulting in enhanced intracellular accumulation of VCR or ACD in MDR cell lines. Cellular uptake (influx) of VCR increased by about 1.5- to 2-fold with DPM in both drug-sensitive and -resistant cell lines. Among the four anticancer agents tested, VCR, ADM, DAU and ACD, DPM rather specifically potentiated VCR and ACD against the MDR cell line, VJ-300. DPM also inhibited photoaffinity labeling of P-glycoprotein by [^{3}H]azidopine, like cepharanthine (Fig. 5). Safa *et al.*[23, 25] have reported inhibition of the [^{3}H]azidopine labeling by verapamil. This reversal action of DPM appears to be exerted through the same drug-efflux-inhibiting mechanism as other MDR-reversal agents such as verapamil and cepharanthine. However, unlike other MDR-reversal agents, DPM does not show a strong effect in reversing ADM- or DAU-resistance in MDR cell lines such as VJ-300. The effect of DPM to potentiate anticancer agents in drug-sensitive and drug-resistant cell lines might involve a unique mechanism.

DPM is a specific inhibitor of transmembrane nucleoside transport[1-3] and this property has been utilized to enhance the cytotoxic action of antitumor antimetabolites by preventing nucleoside salvage and depletion of nucleoside pools.[4-7] In cultured mammalian cell lines, DPM-sensitive and DPM-insensitive transport systems for nucleosides appear to exist.[33] DPM does not inhibit the uptake of 5-FU, but it inhibits the uptake of 5-FUR in both KB and VJ-300 cells, which is in good accordance with a report by Grem and Fischer.[8] Grem and Fischer[9] have further studied the mechanism of potentiation of 5-FU by DPM, and suggested that the metabolism of 5-FU is affected by a selective increase of intracellular fluorodeoxyuridine monophosphate. The combination of 5-FU and DPM shows more than 10-fold potentiation by DPM against KB and VJ-300 cells, but only a 2-fold potentiation against HC-7-5 and HC-7-5/VCR cells. This discrepancy might be due to differences in metabolism of nucleosides or in transport systems among the two cell lines, but it is not yet determined which pathway differs between KB (or VJ-300) and HC-7-5 (or HC-7-5/VCR). DPM appears to potentiate nucleotidic antitumor agents such as 5-FU and other antitumor agents such as VCR or ACD through different mechanisms. Our present study may provide a basis for a unique anticancer combination chemotherapy with DPM, which is useful not only to potentiate some anticancer agents but also to reverse drug-resistance.

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