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The effects of verapamil and a tiapamil analogue, DMDP, on adriamycin-induced cytotoxicity in P388 adriamycin-resistant and -sensitive leukemia in vitro and in vivo

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Summary. DMDP [*N*-(3,4-dimethoxyphenethyl)-*N*-methyl-2-(2-napthyl-*m*-dithane-2-propylamine] a recently developed calcium antagonist analogue, caused a greatly increased intracellular retention of adriamycin and concomitant enhanced cytotoxicity in adriamycin-resistant P388 leukemia cells in vitro. These effects of DMDP were greater than those of another calcium channel blocker, verapamil, and occurred at one-half the dosage levels. Only slight enhancement in adriamycin toxicity was observed for either of these agents in the adriamycin-sensitive parental cell line. However, no significant therapeutic potentiation of adriamycin activity occurred with either verapamil or DMDP treatment in vivo. In vivo maximum DMDP tumor intracellular concentrations, as analyzed by HPLC, were the same in vitro tumor cell levels required to overcome adriamycin resistance. This inability to overcome drug resistance in vivo at acceptable levels of host toxicity is not only a function of maintaining necessary calcium antagonist concentrations in resistant tumor cells.

Introduction

Resistance to chemotherapeutic agents is a common problem experienced in the treatment of cancer. A number of studies have shown that resistant cells accumulate lower drug concentrations than their sensitive counterparts [8, 12]. One mechanism of drug-resistance has been postulated to be due to an enhanced cellular efflux of drug [22]. Overcoming this protective efflux mechanism in drug-resistant cells should lead to enhanced tumor cytoxicity. Verapamil, a calcium channel blocker, has been shown to increase cellular adriamycin retention and cytotoxicity in drug-resistant tumor cells [9], and this effect has also been reported for a number of other calcium antagonists and calmodulin inhibitors [26].

DMDP [*N*-(3,4-dimethoxyphenethyl)-*N*-methyl-2-(2-napthyl-*m*-dithane-2-propylamine)], an analogue of the calcium antagonist tiapamil, has been reported to be more potent than verapamil, 'VRP' [15]. The aim of our investigation was to determine the effects of DMDP on ADR retention and toxicity compared to VRP in P388 adriamycin-resistant (P388/ADR) and -sensitive (P388/O) tumor cells in vitro and in vivo.

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Materials and methods

Chemicals. Adriamycin (ADR) was obtained from ADRIA Laboratories, Columbus, Ohio. Verapamil (VRP) was purchased from SIGMA Chemicals Company, St. Louis, Mo and DMDP was kindly provided by Hoffman-LaRoche, Basel, Switzerland.

Tumor cells. P388/ADR and P388/O murine leukemia cells were supplied by the National Cancer Institute's Frederick Cancer Research Facility, Frederick, Md. These cells have been maintained in our laboratory for 2 years with routine drug resistance checks (once every 2 months). P388/ADR cells have remained 95–100 times more resistant to ADR than P388/0 cells throughout this period.

Cell culture. Tumor cells were maintained in plastic 75-cm^2 (250 ml) T-flasks (Becton-Dickinson Labware, Oxnard, Calif) in Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal bovine serum [Grand Island Bottling Co. Labs (GIBCO), Chagrin Falls, Ohio], 1% penicillin and streptomycin (GIBCO, Grand Island, NY) and $10^{-5}M$ concentration of 2-mercaptoethanol (Fisher Scientific Co., Fair Lawn, NJ). Oxygen free radical scavengers may be involved in the cytotoxic effects of ADR (1). Some studies have reported that oxygen radical scavengers may decrease the cytotoxic effects of ADR (6). 2-ME can act as an oxygen radical scavenger and could alter the cytotoxic effects of ADR. However, no effects of 2-ME on ADR ED_{50} were found, and so the culture studies employing 2-ME did not interfere with the interpretation of the results. In addition Tsuruo used 2-ME in his *in vitro* studies (27). Cells were grown in an 37° C humidified atmosphere of 5% CO_2, in air. Each cell line was resupplied with fresh medium every 24–28 h, maintaining a concentration of $4.0–6.0 \times 10^5$ cells/ml. Under these conditions exponential growth, with a cell doubling time of approximately 24 h, occurred for both cell lines.

Drug treatment. Each cell line was washed in phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS w/out) (GIBCO, Grand Island, NY) and resuspended in 75 cm^2 T-flasks at a concentration of 5×10^5 cells/ml. The flasks were then placed in a CO_2 incubator and the cells left to equilibrate for 1 h before experimentation. VRP or DMDP (at a final concentration of 6.1 μM or 3.1μM, respectively) was added to the appropriate flasks and incubated for 5 min prior to addition of ADR (at $5 \times 10^{-6}M$). The flasks

were then placed in a CO_2 incubator on a rocker platform, and at 1, 6, 24 and 48 h thereafter cell samples were removed and assayed for ADR and protein content. Samples were also removed at each time interval to determine cell viability by visual scoring for cellular exclusion of 0.1% trypan blue strain.

A number of methods have been used to assess cell viability, including cell doubling time, thymidine labeling index, chromium release, in vitro colony formation and dye exclusion. Although in vitro colony formation may be a reliable index of cell viability [17], dye exclusion methods are simple, quick and reliable for comparative studies such as that described in the present report.

The short-term ADR/long-term DMDP in vitro P388/ADR experiments were conducted in the same manner as the aforementioned drug treatment experiments, except that after an initial 2-h treatment with ADR (at $5 \times 10^{-6} M$) the cells were washed twice in 37° C PBS w/out and then resuspended in the presence or absence of DMDP (3.1 μM). Net intracellular ADR accumulation and cell viability were then determined for different times during a 48-h treatment period.

High-pressure liquid chromatography Assay. HPLC analysis of DMDP associated with plasma and cells (in vitro and in vivo) was determined using a μBondapak phenyl C-18 reversed phase column with acetonitrile/H_2O/ammonium hydrochloride (45:54.5:0.5% w/v) buffered to pH5 with formic acid as the mobile phase. Cells were washed twice with PBS w/out, extracted with methanol (0.4 ml/10^6 cells) and centrifuged at $3000 \times G$ for 15 min. The flow rate was 2 ml/min and the DMDP peak assayed at 229 nm had a retention time of 13 min.

Adriamycin equivalent assay and protein assay. For the assays duplicate cell samples (2.5×10^6 cells) were removed, placed in 15 ml disaposable centrifuge tubes (Falcon, Oxnard, Calif) and spun down on an IEC CRU-5000 centrifuge at 450 g for 5 min at 4° C. The samples were washed twice in 5 ml cold PBS w/out by the same procedure. The cell pellet was then resuspended in 1.0 ml H_2O and left overnight, in the dark, to lyse the cells. All experimental procedures and assays were performed in a light-attenuated environment because of the light sensitivity of ADR. After 24 h two 0.1 ml aliquots were removed from each sample – one for the fluorometric assay of ADR and the other for a protein assay [16] with slight modifications [20]. For the fluorometric assay the samples were suspended in 1.9 ml methanol (Fisher Scientific Co., Fair Lawn, NJ) and read on a Perkin-Elmer 650-10S fluorescence spectrophotometer (excitation wavelenght at 468 nm, emission wavelength at 550 nm and 585 nm, slit at 2 nm, and sensitivity at 3.5). The two emission wavelength values (the two fluorometric peaks of ADR) from each sample were averaged and compared with a standard ADR curve to determine the concentration of ADR. The Lowry protein assay was performed using a Perkin-Elmer Coleman 124 double-beam spectrophotometer at a wavelength setting of 750 mμ and compared with an albumin standard (Bio-Rad Labs, Rockville Center, NY). The ADR equivalent amount was then correlated to the protein content in the cell sample (nanograms of ADR/micrograms of protein).

The efficience of recovery of ADR from cells and the quenching of drug fluorescence was also investigated. Tumor cells were treated with a known amount of ADR prepared for assay as above. Aqueous samples 0.1 ml in volume were either read directly on a spectrophotometer or had a known amount of ADR added and were then read. The results showed that the recovery of ADR was greater than 90% with only a 6%–8% quenching effect observed. These cell assays resulted in higher recoveries and lower quenching than tissue extracts, because the entire cell sample was extracted and measured. No separation of tumor fragments from the measured sample was required since the actual total cell volume was very small compared with the extraction volume.

Animal studies. DBA/2J female mice (Jackson Laboratory, Bar Harbor, Maine) were innoculated i.p. with 10^5 tumor cells on day 0, with treatment beginning on day 1. VRP therapy consited of i.p. injections of VRP (at 50 or 75 mg/kg) and/or ADR (at 1 mg/kg) for 10 consecutive days as described by Tsuruo et al. [26]. DMPD therapy was administered according to two schedules: (1) DMDP was injected i. p. once an hour for 4 h with ADR (10 mg/kg) injected i. p. between the 3rd and 4th DMDP injection; (2) DMDP was administered by continuous i.v. infusion, using a Harvard pump (Harvard Apparatus, South Natick, Mass), for 3 days (at 750 mg/kg per day) with ADR (at 1.0 or 3.0 mg/kg per day) injected i.p.

Results

Toxicity of VRP and DMDP

The in vitro toxicity of VRP and DMDP was determined for the P388/ADR and P388/O cell lines (Fig. 1). The ED_{50} (effective dose resulting in 50% cell viability) for P388/ADR and P388/O cells exposed for 48 h to VRP was 82 $\mu M \pm 1.7$ and 94 $\mu M \pm 2.1$, respectively, whereas for DMDP treated cells it was 12 $\mu M \pm 0.7$ and 16 $\mu M \pm 1.0$, respectively. At final concentrations of 6.1 μM for VRP and 3.1 μM for DMDP, which were used in all the experiments, no effect on either cell viability or growth was observed for either P388/ADR or P388/O cells.

Potentiation of ADR cytotoxicity by VRP and DMDP

The cytotoxicity of ADR to P388/ADR cells was enhanced greatly after treatment with VRP or DMDP (Fig. 2). There were 3- and 11-fold increases in cytotoxicity to P388/ADR cells exposed for 24 and 48 h, respectively, to VRP + ADR, whereas DMDP + ADR treatment resulted in 4- and 19-fold enhancements, respectively, compared with ADR only treatment (Fig. 2A). Neither DMDP nor VRP treatment enhanced ADR cytotoxicity against P388/O cells (Fig. 2B).

Enhanced intracellular accumulation of ADR by VRP and DMDP

The intracellular accumulation of ADR in P388/O and P388/ADR cells is shown in Fig. 3. ADR retention in P388/ADR cells increased markedly on exposure to VRP or DMDP. Treatment of P388/ADR cells with VRP + ADR (Fig. 3A) resulted in 1.8-, 3.7- and 4.4-fold enhancements of drug accumulation in comparison with ADR only treated cells at 1, 6 and 24 h, respectively ($P < 0.05$). There were 2.2-, 4.5- and 5.5-fold increases in intracellular drug retention for DMDP + ADR-treated

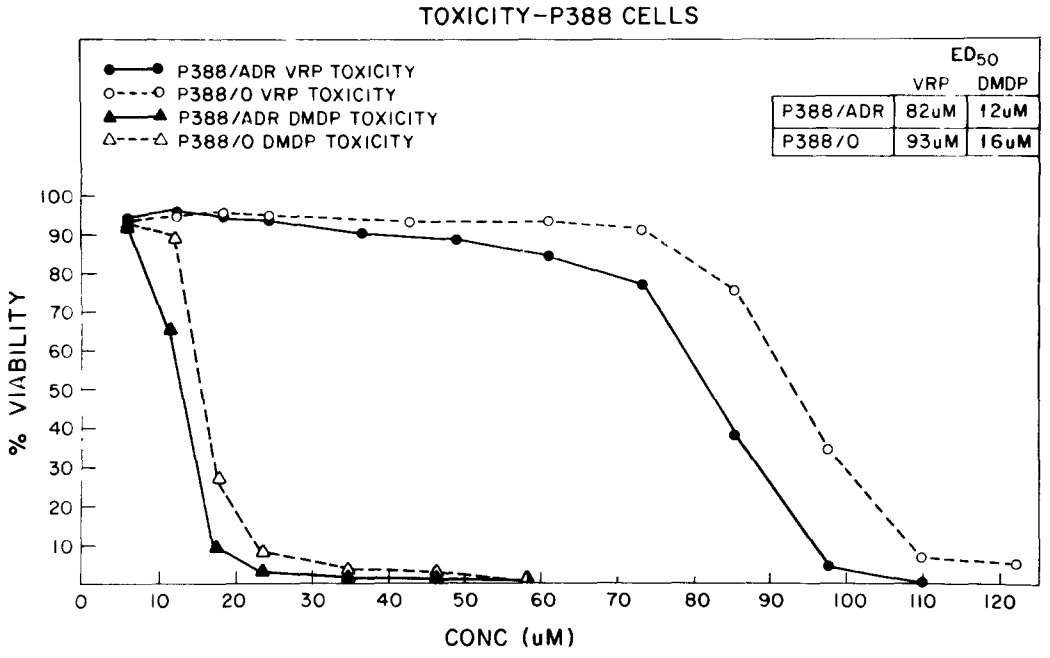

Fig. 1. Toxicity of VRP or DMDP in P388/ADR and P388/O cells. Cells were incubated for 48 h with increasing concentration of VRP or DMDP as follows P388/ADR cells with VRP (●———●) or DMDP (O-----O); and P388/O cells with VRP (▲———▲) or DMDP (△------△). Cell viability was determined using 0.1% trypan blue staining

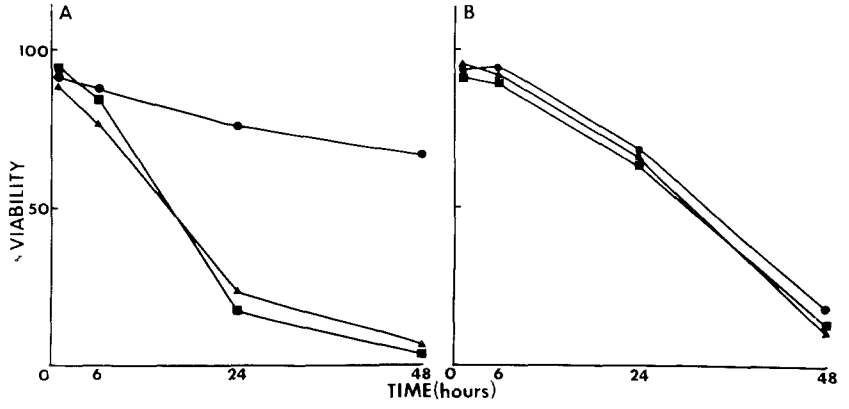

Fig. 2. Viability of P388/ADR (A) or P388/O (B) cells in the presence of ADR (5μ*M*) with or without VRP (6.1μ*M*) or DMDP (3.1μ*M*). Cells were incubated for different times up to 48 h, with VRP+ADR (▲), DMDP+ADR (■) or ADR alone (●), and viability was determined using 0.1% trypan blue staining

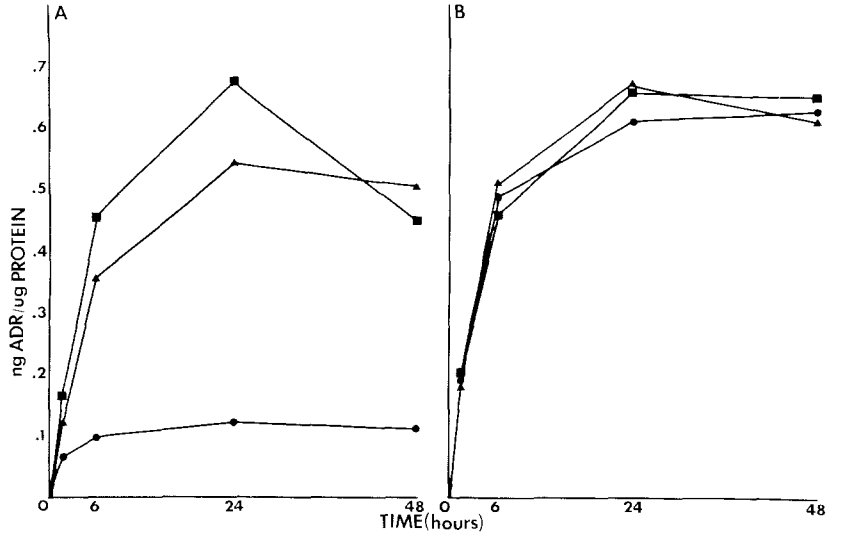

Fig. 3. Net accumulation of ADR (5μ*M*) by P388/ADR (A) or P388/O (B) cells in the presence or absence of VRP (6.1μ*M*) or DMDP (3.1 μ*M*). Cells were incubated for different times, up to 48 h, with VRP+ADR (▲) DMDP+ADR (■) or ADR alone (●) and ADR content determined using fluorescent spectroscopy

Table 1. Net uptake [ng ADR/ug protein (± SEM)] and viability as percentage (± SEM) for P388/ADR cells treated with DMDP (3.1 μM) and ADR (5×10^{-6} M) for 2 h, washed twice in PBS w/out, and then incubated for 48 h in the presence or absence of DMDP (3.1 μM)

Time (h)	Uptake (without DMDP)	Viability	Uptake (with DMDP)	Viability
0	0.196 (0.013)	92.8 (1.0)	0.196 (0.013)	92.8 (1.0)
1	0.153 (0.011)	90.9 (0.8)	0.171 (0.020)	91.8 (1.6)
6	0.074 (0.006)	80.1 (1.2)	0.165 (0.017)	68.3 (1.7)
24	0.052 (0.009)	65.5 (1.6)	0.167 (0.014)	12.6 (1.5)
48	0.033 (0.006)	60.7 (1.4)	0.164 (0.012)	3.0 (0.5)

Table 3. Mean survival time in days (± SEM) and [% increased life span] for P388-tumor-bearing DBA/2J mice (10^5 cells i.p. on day 0) treated i.p. once an hour for 4 h with various concentrations of DMDP, with ADR (10 mg/kg) given between the 3rd and 4th injections of DMDP

Group	P388/ADR	P388/O
Control	11.4 (0.4)	11.0 (0.4)
ADR	9.6 (0.5) [−15.8]	21.6 (1.0) [+96.4]
DMDP (15 mg/kg)	12.2 (0.4) [+7.0]	10.0 (0.6) [−9.1]
DMDP (15 mg/kg) + ADR	10.3 (0.9) [−9.7]	19.0 (4.7) [+72.7]
DMDP (30 mg/kg)	11.0 (0.6) [−3.5]	10.2 (0.2) [−7.3]
DMDP (30 mg/kg) + ADR	10.3 (0.3) [−9.7]	17.0 (3.8) [+54.6]
DMDP (60 mg/kg)	10.5 (0.3) [−7.9]	10.6 (0.2) [−3.6]
DMDP (60 mg/kg) + ADR	7.5 (2.6) [−34.2]	7.0 (1.0) [−36.4]

Table 2. Mean survival time in days (± SEM), [% increased life span] and the median survival for P388 tumor-bearing mice (10^5 cells i.p. on day 0) treated i.p. on days 1 – 10 with VRP and/or ADR

Group	P388/O			P388/ADR		
	Mean	%ILS	Median	Mean	%ILS	Median
Control	12.3 (0.2)		12	13.4 (0.2)		13
VRP (75 mg/kg)	12.0 (0.6)	[−2.4]	11	13.7 (0.3)	[+2.2]	14
ADR (1 mg/kg)	25.7 (2.5)	[+108.9]	25	14.3 (0.3)	[+6.7]	14
VRP (50) + ADR[a]	20.6 (1.2)	[+67.5]	20	16.6 (0.3)	[+23.9]	16
VRP (75) + ADR[a]	21.7 (1.0)	[+76.5]	20	16.8 (0.4)	[+25.4]	16

[a] VRP at either 50 mg/kg or 75 mg/kg; ADR at 1 mg/kg

P388/ADR cells compared with those treated with ADR only ($P < 0.05$). ADR retention in P388/0 cells (Fig. 3 B), for all forms of treatment, showed no statistically significant differences at all the time intervals (P values ranged from 0.5 to 0.8).

Short-term ADR treatment concurrent with long-term DMDP treatment

Table 1 shows the results of experiments in which P388/ADR cells were incubated for 2 h only with ADR ($5 \times 10^{-6} M$) and for 48 h with DMDP (3.1μM). In the absence of DMDP P388/ADR cells lost 62% of their ADR by 6 h with this loss progressing to 73% and 83% by 24 and 48 h, respectively. Cells incubated with DMDP lost only 16% of drug by 6 h and suffered no further losses for up to 48 h. Cell viabilities in the absence of DMDP were 66% and 61% at 24 and 48 h, respectively, whereas DMDP-treated cells had a 13% and 3% viability. These results were identical to those obtained from a 48-h exposure to both DMDP and ADR (17% and 4% at 24 and 48 h respectively) demonstrating that the same cytotoxicity from ADR treatment in vitro can be achived at only 5% of the original ADR exposure time, if intracellular ADR levels are maintained due to the presence of DMDP.

In vivo therapy of P388 cells with VRP and ADR

The results are shown in Table 2. P388/O tumor bearing mice demonstrated a greatly increased mean survival time (MST) after ADR therapy compared to the untreated mice. All groups of P388/O tumor-bearing mice treated with ADR displayed a statistically significant increase in MST ($P < 0.05$) in comparison with the control group [25.7, 20.6, 21.7 and 12.3 days for ADR, VRP (50 mg/kg) + ADR, VRP (75 mg/kg) + ADR and the control group, respectively]. No additional enhancement in MST resulted from the VRP + ADR treatment of P388/O tumor-bearing mice in comparison with mice treated with ADR alone. In addition, surival of mice treated with VRP + ADR was less than mice treated with ADR alone.

Table 4. DMDP and ADR levels (nanomoles/10^6 cells) were determined in P388/O and P388/ADR cells by HPLC analysis. P388-tumor-bearing DBA/2J mice (10^5 cells i.p. on day 0) were treated i.p. once an hour for 4 h with DMDP (60 mg/kg/h × 4), with ADR (10 mg/kg) given between the 3rd and 4th injections of DMDP. Cells were removed for analysis 1 hour after the last DMDP injection

	P388/O	P388/ADR
DMDP	0.71 (±0.11)	0.58 (±0.17)
ADR	0.46 (±0.02)	0.28 (±0.02)

P388/ADR tumor-bearing mice treated with ADR showed a slight increase in MST ($P < 0.05$) compared with the control group. A larger increase in MST was observed in mice treated with both VRP and ADR. The groups pretreated with VRP demonstrated a significant difference ($P < 0.05$) compared with the ADR only group. However, these increases in survival time were less than those reported by Tsuruo et al. [26].

In vivo therapy using DMDP and ADR

DMDP therapy using multiple i.p. injections or continuous i.v. infusion was performed on P388 tumor-bearing

mice. The effects of i. p. injections of DMDP and ADR are shown in Table 3. DMDP+ADR did not increase the survival time of P388/O tumor-bearing mice over that with ADR only treatment. A combined toxicity from DMDP (60 mg/kg, Q 1 h×4)+ADR (10 mg/kg) treatment resulted in lower survival times in both P388/ADR and P388/O tumor-bearing mice compared with untreted mice. The mean plasma concentration of DMDP, measured using HPLC (High-pressure liquid chromatography) was 5.4 (±0.6) nmoles/ml plasma, whereas the concentration in P388/0 and P388/ADR cells from the peritoneal cavity was 0.71 (±0.11) nmoles/10^6 cells and 0.58 (±0.17) nmoles/10^6 cells, respectively (samples were removed 1 hour after the last DMDP injection for analysis). DMDP and ADR levels for tumor cells treated in vivo are shown in Table 4.

P388 tumor-bearing mice were also treated with a continuous i.v. infusion of DMDP at 750 mg/kg per day and i.p. push injections of ADR (1.0 or 3.0 mg/kg per day) over a 3-day period. No increased antitumor activity resulted from the continuous infusion of DMDP alone or in combination with ADR. Infused mice demonstrated a mean DMDP plasma concentration of 4.7 (±0.6) nmoles/ml plasma (samples were removed for analysis immediately after the infusions were discontinued). It is evident that a continuous i.v. infusion of DMDP achieved the same plasma concentration as that of i.p. injection treatments. However, less than 0.3 nmoles DMDP accumulated per 10^6 tumor cells (i.p.). This most likely resulted from the reduced access of DMDP to the peritoneal cavity when administered through the i.v. route.

Discussion

The chemotherapy of cancer is limited because of various factors, including the selection and growth of drug-resistant tumor cells [19, 21]. This problem is often compounded by development of cross-resistance to other useful chemotherapeutic agents, which severely limits treatment alternatives [2, 4]. The mechanism of resitance to anthracyclines in many tumor systems is postulated to be an enhanced outward transport of drug [5, 23]. Overcoming increased drug efflux in resistant cells could lead to a larger therapeutic effect, resulting in a better prognosis. Therapeutic potentiation was reported to occur when tumor cells were treated with anthracyclines and the calcium channel blocker verapamil [24].

In our in vitro studies the resistant cells treated with VRP showed a marked enhancement in ADR retention and cytotoxicity, resulting in a greater than 4-fold increase in intracellular ADR. In P388/ADR cells treated with VRP+ADR cytotoxicity was higher at 24 and 48 h than in P388/O cells. A recently developed calcium antagonist, DMDP, was tested in vitro as to its effect on the antineoplastic activity of ADR. Our studies showed that DMDP (at half the dosage of VRP) produced an equal, cytotoxic activity against P388/ADR cells compared to VRP. Cell viability at 24 and 48 h for VRP+ADR was 23.1% and 6.2% respectively, whereas DMDP treatment resulted in 18.5% und 3.5% viability. Neither of these calcium antagonists potentiated the antineoplastic activity of ADR against the P388/O cells. The results from this study, and other investigations [10, 25], suggests that the P388/ADR cells have drug efflux capabilities that the sensitive cells lack.

P388/ADR cells were also incubated in vitro with a short exposure to ADR and a long exposure to DMDP to determine the effectiveness of this treatment. DMDP treatment maintained 84% of the original intracellular ADR concentration for up to 24 h whereas the absence of this agent resulted in a 73% loss of intracellular drug. P388/ADR cells treated for 2 h with ADR and 48 h with DMDP resulted in a cytotoxicity equal to that obtained with a 48-h treatment with both agents, even though the intracellular ADR was 4-fold less. Incubation of P388/ADR cells in the presence of non-toxic levels of DMDP for a prolonged period therefore enhanced retention and cytotoxicity of ADR incubated with the cells for a relatively short time period. It might be suggested that if DMDP levels which enhanced prolonged retention of a single cytotoxic dose of ADR could be maintained in vivo significant improvements in cell kill would occur.

The effects of the calcium antagonists on the antitumor activity of ADR in vivo showed that VRP+ADR therapy in P388/ADR tumor-bearing mice resulted in only a slight increase in survival time compared with ADR treatment. This result was well below those published by Tsuruo [24]. The small increase in life span was also much below the large increase in cytotoxicity seen in vitro in P388/ADR cells with VRP treatment. The poor in vivo results were attributed to the inability to attain intracellular VRP concentrations necessary to increase the antitumor activity of ADR. A further study using a 5-day continuous i.v. infusion of VRP and ADR in P388/ADR tumor-bearing mice also demonstrated no enhanced antitumor activity [18].

In vivo therapy with DMDP administered i.p. also did not result in an increase in ADR toxicity to P388/ADR cells. HPLC analysis of intracellular tumor cell levels indicated that DMDP was concentrated within the cells >100 times compared to the plasma concentration (the nature of the cellular binding sites are not known at present). Most other studies have relied upon the plasma concentration of the calcium antagonists as an indication of the tumor cells concentration. Our results demonstrate that DMDP concentrates within the tumor cells in vivo at higher levels than those necessary to reverse drug resistance in vitro. The absence of an increased antitumor response in vivo has been attributed previously to the lack of maintaining necessary plasma calcium antagonist concentrations. However, our study demonstrates that resistant tumor cells concentrate DMDP at levels that should lead to an increased cytotoxicity in drug-resistant tumor cells and suggest that there are additional mechanisms involved in reversing drug resistance other than high intracellular concentrations of DMDP. This lack of an effect could be due to biochemical changes or modulations in the sensitivity of the in vivo tumor cells, such as sequestering the DMDP from its intended target, that make them less sensitive to the effects of DMDP that is observed in vitro.

From a number of studies it appears that the calcium antagonists increase net anthracycline accumulation in drug-resistant tumor cells through an impairment of the outward transport of drug [11, 27]. The mechanism of this inhibition remains unclear. A recent study reported that VRP increased anathracycline accumulation in drug-resistant tumor cells without affecting calcium transport, suggesting that VRP may function through a competitive-type inhibition of anthracycline efflux [14]. A glycoprotein of

180000 molecular weight has been found in the P388/ADR tumor cell line [13]. The calmodulin inhibitor trifluoperazine and the calcium antagonist VRP, when incubated with Chinese hamster lung cells resistant to ADR have been reported to cause an increase in drug accumulation concomitant with enhancement of the phosphorylation of a glycoprotein (180 KD) present only in the resistant cells [3]. The increased sensitivity of P388/ADR cells to adriamycin could result from a similar glycoprotein phosphorylation, from VRP or DMDP, with no effect on calcium ion influx. A recent study has shown that VRP binds to a large polypeptide chain of 170000 molecular weight found in the receptor site of the calcium channel [7]. This suggests that VRP and DMDP may increase sensitivity in drug-resistant cells through affinity binding to these "resistance" glycoproteins. This would inactivate the drug efflux mechanism of these cells, resulting in higher intracellular ADR levels and concomitant enhanced cytotoxicity.

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