Anti-Cancer Drugs, 4, pp. 173–180

D-Verapamil downmodulates P170-associated resistance to doxorubicin, daunorubicin and idarubicin

Daniela Damiani, Mariagrazia Michieli, Angela Michelutti, Cristina Melli, Michela Cerno and Michele Baccarani

Chair of Anatomy and Chair of Hematology, Institute of Experimental and Clinical Morphological Research, Udine University School of Medicine, Udine, Italy

Verapamil (VRP) is an effective modulator of P170-associated multidrug resistance (MDR), but its clinical application is limited by cardiovascular side-effects. The D-isomer of VRP (D-VRP) is 10 times less active than the racemic mixture on the cardiovascular system, but retains a MDR modulating activity. Daunorubicin (DNR) and doxorubicin (DX) are two anthracyclines whose cytotoxicity is strongly related with the expression of P170, while their respective lipophylic derivatives idarubicin (IDA) and iododoxorubicin (IDX) are less P170-dependent. We studied the effect of D-VRP on intracellular retention and on the cytotoxicity of these four anthracyclines in two MDR cell systems (LOVO and CEM) by flow cytometry and by a microcultured tetrazolium colorimetric assay (MTT). We found that in MDR cells D-VRP increased intracellular anthracycline concentration and increased the cytotoxicity of DNR, IDA and DX but not of IDX. The effect of D-VRP was dose-related, but it was already consistent at D-VRP concentrations that can be readily maintained *in vivo* (2–3 μM). These data suggest that at a clinically tolerable concentration D-VRP can downmodulate the resistance to DNR and DX and can restore full sensitivity to IDA.

Key words: Anthracycline, chemotherapy, resistance.

Introduction

The *mdr-1* gene codes for a 170 kDa transmembrane glycoprotein (P170) which modulates the intracellular concentration of several cytotoxic agents, by enhancing the efflux of the drugs out of the cells.[1–4] Amplification or overexpression of the gene leads to reduced sensitivity to cytotoxics, including anthracyclines, anthracenedione derivatives, Vinca alkaloids, epipodophylline derivatives and others, a phenomenon that was described by the term of pleiotropic or multidrug resistance (MDR).[1,5]

The effects of P170 on drug efflux can be modulated by many calcium channel-blocking agents and other substances.[6–16] Verapamil (VRP) was the first drug that was shown to be able to restore drug sensitivity in MDR cells[13,15] and was first tested in a clinical setting to increase treatment efficacy in resistant human tumors.[17–20] A major problem of the clinical application of VRP was that since VRP is a calcium-blocking agent, hypotension and heart block would prevent reaching and maintaining *in vivo* the concentrations which are required to reverse MDR *in vitro*.[21–23] The cardiovascular effects of VRP depend on its calcium-blocking activity, but the modulatory effects of VRP on P170 do not depend on calcium blocking.[24,25]

VRP is a racemic mixture of equal amounts of two enantiomers, and the L-isomer is about 10 times more potent than the D-isomer (D-VRP) with regard to calcium channel blocking and cardiovascular effects.[21,23] Since the multidrug reverting effects of VRP do not depend on calcium blocking,[24,25] D-VRP is likely to be a better candidate than the racemic mixture for the purposes of downmodulating MDR in a clinical setting. In order to provide preclinical data for the application of D-VRP to tumor treatment, we studied the effect of D-VRP on the cell retention of, and on the cell sensitivity to, the major anthracyclines daunorubicin (DNR) and doxorubicin (DX) and their lypophylic derivatives idarubicin (IDA) and iododoxorubicin (IDX).

Materials and methods

Drugs

DNR, DX and IDA were purchased by Farmitalia-Carlo Erba, Italy. IDX was a gift from Farmitalia-Carlo Erba, Italy. All drugs were dissolved in

Work supported by CNR, Finalized Project Clinical Application of Oncologic Research, contract no. 92.02128.PF39 and by AIRC, Milano. A.M. is an AIRC fellow.

Correspondence to D. Damiani, Cattedra di Ematologia, Dipartimento di Ricerche Cliniche e Morfologiche, P. le S. Maria della Misericordia, 33100 Udine, Italy. Tel: (+39) 432-559662-4. Fax: (+39) 432-559661.

distilled water at 100 μg/ml and aliquots were stored at −20°C. D-VRP was a gift from Knoll Farmaceutici SpA, Italy. D-VRP was dissolved in methanol and aliquots were stored at −20°C.

Cell lines

We used the colon adenocarcinoma cell line LOVO 109[26] and its MDR DX-selected subline LOVO DX,[27] and the T cell acute lymphocytic leukemia cell line CCRF CEM (CEM)[28] and its MDR vinblastine (VLB)-selected subline CEM VLB.[29] Both LOVO DX and CEM VLB, but not LOVO 109 and CEM, were previously shown to carry *mdr-1* gene amplification[30,31] and this was confirmed in our laboratory (by the kind cooperation of Dr Giacca, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy).[32] All cell lines were cultured at 37°C in a humidified atmosphere of 5% CO_2 and maintained in exponential growth in RPMI 1640 (Biochem KG) supplemented with 10% heat-inactivated fetal calf serum (Biochem KG), 2 mM glutamine solution, 100 U/ml penicillin and 100 μg/ml streptomycin (Biochem KG). The medium of the resistant subline LOVO DX was always supplemented with 100 ng/ml of DX and the medium of the resistant subline CEM VLB was always supplemented with 100 ng/ml of VLB, until 3 days before the experiments. For all studies, cells were harvested during exponential growth, washed twice in medium and resuspended at the required concentration. In our hands, when LOVO DX and CEM VLB were fixed in periodate lysine paraformaldehyde (PLP) and permeabilized in saponine 0.02% for immunofluorescence procedures, a very strong membrane-associated fluorescence in more than 80% of the tested cells was obtained using the P170-directed, MRK-16 antibody, while LOVO 109 and CEM showed no staining at all or a low staining intensity in less than 5% of the cells. The mean fluorescence intensity (MFI) determined as the ratio of the MFI of the sample and the isotypic control was always less than 4 in the two parental cell lines LOVO 109 and CEM while it ranged between 14 and 16 in CEM VLB, and between 28 and 32 in LOVO DX. The methods used for MDR studies were described elsewhere.[32]

Drug sensitivity assay

Cell growth in the presence or absence of drugs was determined using the MTT-microcultured tetrazolium colorimetric assay of Mosmann[33] with minor modifications, as described elsewhere.[32] Briefly, anthracyclines and D-VRP were added at the required concentration after 48 h incubation in microplates. Cell growth and growth inhibition were evaluated after a 7 day incubation in continuous drug exposure at 37°C in humidified atmosphere containing 5% CO_2. MTT solution was added at 5 mg/ml and DMSO was used as the MTT formazan-products solvent. Optical density (OD) was read at 540 nm using a microcultured plate reader (LP400 Diagnostic Pasteur). Results were expressed in terms of percentage of growth as compared with the control. Controls were provided by cells plus D-VRP solvent, cells plus anthracyclines and D-VRP solvent or cells plus D-VRP as appropriate. From each OD, appropriate background values were subtracted (culture medium alone or culture medium with D-VRP). At the tested doses, D-VRP alone gave a growth inhibition ranging between 5 and 30% in all cell lines.

All the experiments were performed at least in triplicate and the results were the mean of at least three values. Standard deviation were always 10% or less.

The ID_{50}, i.e. the drug concentration which inhibited cell growth to 50% compared with the control, was calculated as the logarithm of the drug concentration at which the best line fitted by eye to the data would cross a surviving fraction of 0.5. The DX, IDX, IDA and DNR ID_{50}s of the cell lines and the resistance index (RI), i.e. the ratio between the ID_{50} of the MDR positive lines and the respective ID_{50} of the parental MDR negative cell line, are shown in Table 1.

Table 1. ID_{50} of the MDR (LOVO DX and CEM VLB) and of the parental, non-MDR cell lines (LOVO 109 and CEM) in the absence of D-VRP

	ID_{50} (ng/ml)			
	DNR	IDA	DX	IDX
LOVO DX	410.0	13.0	460.0	12.0
LOVO 109	10.0	2.5	15.0	7.5
RI	41.0	5.2	30.6	1.6
CEM VLB	78.0	3.2	100.0	3.0
CEM	3.0	1.2	4.0	2.2
RI	26.0	2.7	25.0	1.4

Each number represents the mean of three values. Standard deviation is not reported but was always 10% or less. The RI was calculated by dividing the ID_{50} of the MDR line by the ID_{50} of the parental line, and it was much lower for IDA than for DNR, and for IDX than for DX.

Intracellular drug concentration

Intracellular drug concentration was determined by flow cytometry with a FACScan (Becton-Dickinson) equipped with an argon laser tunned at 488 nm. Lysis II software package (Becton-Dickinson) was used to generate FL2 histograms to calculate the means of the linear fluorescence intensity distribution and to generate the forward scatter (FSC) histograms to calculate the relative cell volume. Results are expressed as the normalized mean fluorescence index (NMFI) as a measure of relative intracellular drug accumulation, where:

$$NFMI = (FL2/FSC) - (FL2_0/FSC_0)$$

NMFI = mean fluorescence channel number/mean FSC channel number with drug − mean fluorescence channel number/mean FSC channel number without drug

in accordance with Luk.[34]

Cells at a concentration of 2.5×10^6/ml were incubated in RPMI supplemented with 10% fetal calf serum and the four anthracyclines at concentrations ranging between 50 and 3000 ng/ml for 1, 2 and 3 h. After 1, 2 and 3 h cells were washed twice in ice-cold phosphate-buffered saline (PBS) at pH 7.4, resuspended in 1 ml ice-cold PBS and immediately assayed for drug-associated fluorescence. Controls were performed by cells incubated in the absence of drugs but in the presence of solvents. Results are shown in Figure 1 for DNR. For the other anthracyclines, they were similar. Because of the good correlation between drug dose exposure and fluorescence intensity (NFMI), for D-VRP studies only an exposure to 1000 ng/ml of all the tested anthracyclines was used.

To evaluate D-VRP effects, 10 μM D-VRP was added to the complete medium (RPMI 1640 plus fetal calf serum, plus anthracyclines 1000 ng ml). Controls were cells plus solvents, cells plus anthracyclines or cells plus D-VRP as appropriate.

Results

Cytotoxicity assay (MTT)

Figures 2 and 3 show that the addition of D-VRP increased the cytotoxicity of DNR and IDA in the MDR LOVO DX cell line, but not in the parental, non-MDR LOVO 109 line. Similar results were obtained with DNR and IDA in the CEM cell system, and with DX and IDX in both cell systems

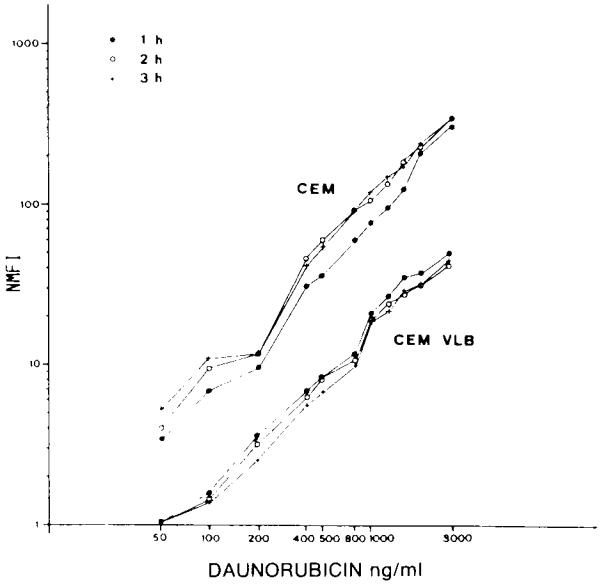

Figure 1. Daunorubicin content expressed in NMFI according to DNR incubation concentration after 1, 2 or 3 h exposure in the CEM system lines. CEM is the parental sensitive cell line, CEM VLB is the resistant MDR positive cell subline. There is a good correlation between drug dose exposure and fluorescence intensity expressed in terms of NMFI.

(LOVO and CEM). Results are summarized in Tables 1–4. Table 1 shows the ID_{50} for the two cell systems and the four tested drugs, in the absence of D-VRP. Table 2 shows the D-VRP-dependent decrease of anthracycline ID_{50} in the two MDR lines. Tables 3 and 4 show the effects of increasing concentration of D-VRP on the RI to the four anthracyclines in the LOVO system (Table 3) and in the CEM system (Table 4). The main conclusions of these experiments were that (i) IDA was more was more cytotoxic than DNR and IDX was more cytotoxic than DX in all cell lines, but the difference between IDA and DNR and between IDX and DX was much greater in the MDR lines (LOVO DX and CEM VLB) than in the parental, non-MDR ones (LOVO 109 and CEM), (ii) D-VRP increased anthracycline cytotoxicity only in the two MDR lines, (iii) D-VRP increased anthracycline cytotoxicity in a concentration-related manner, and (iv) D-VRP increased the cytotoxicity of DNR and DX more than that of the respective derivatives IDA and IDX.

Intracellular drug retention

To determine the effect of D-VRP on intracellular drug retention, cells were incubated with the tested

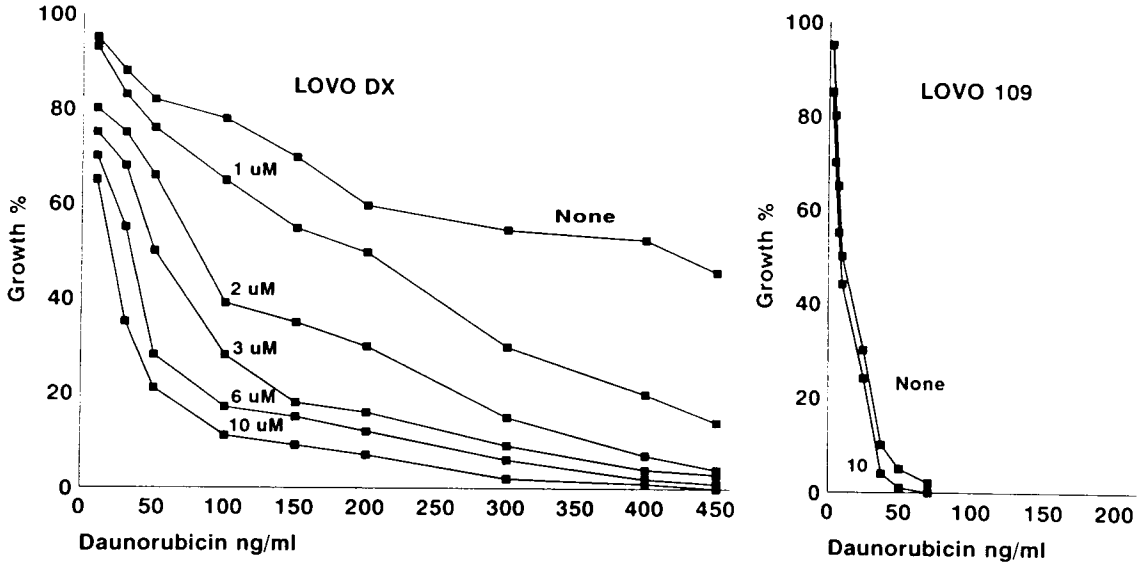

Figure 2. Effect of DNR alone (None) or combined with D-VRP 1, 2, 3, 6 and 10 μM on the growth of the drug resistant LOVO DX and on the growth of the parental sensitive LOVO 109 cell line. Growth is represented as percentage of control cell growth in cultures containing no drugs but all solvent or no drug but D-VRP as appropriate. Each point represents the mean of three values. Standard deviation was always 10% or less. D-VRP combined with DNR had a dose-dependent growth inhibition effect on LOVO DX but not on LOVO 109. For LOVO 109 only the effect of D-VRP 10 μM was represented. All the other D-VRP concentrations tested had a decrease or no effect on cell growth.

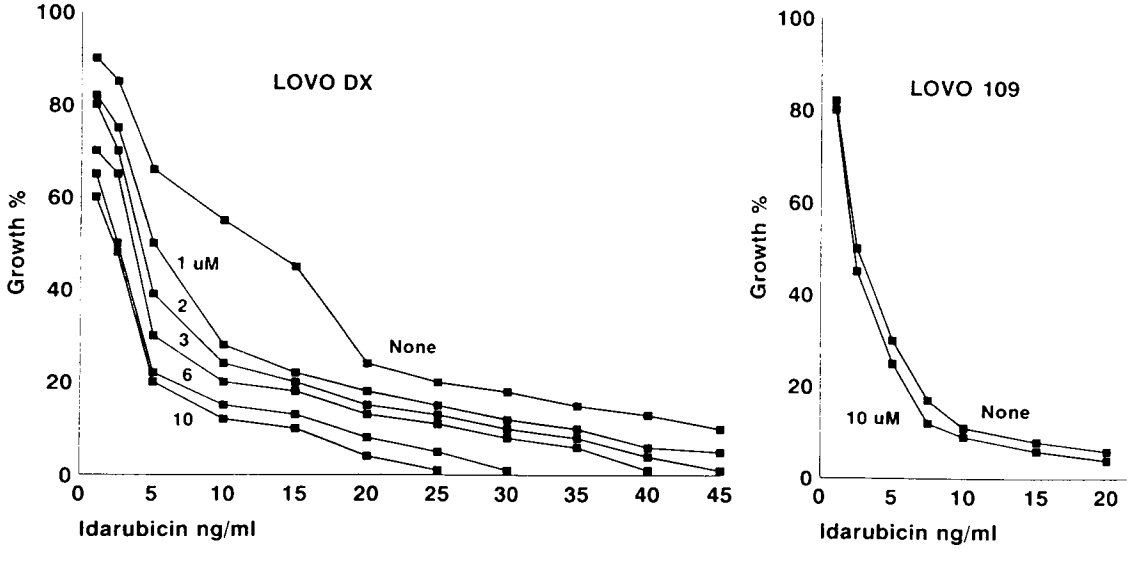

Figure 3. Effect of IDA alone (None) or combined with D-VRP 1, 2, 3, 6 and 10 μM on the growth of the drug resistant LOVO DX and on the growth of the parental sensitive LOVO 109 cell line. Growth is represented as percentage of control cell growth in cultures containing no drugs but all solvent or no drug but D-VRP as appropriate. Each point represents the mean of three values. Standard deviation was always 10% or less. D-VRP combined with IDA had a dose-dependent growth inhibition effect on LOVO DX but not on LOVO 109. For LOVO 109 only the effect of D-VRP 10 μM was represented. All the other D-VRP concentrations tested had a decrease or no effect on cell growth.

Table 2. ID_{50} with and without D-VRP for the two MDR cell lines

	D-VRP (μM)	DNR		IDA		DX		IDX	
		ID_{50} (ng/ml)	ratio	ID_{50} (ng/ml)	ratio	ID_{50} (ng/ml)	ratio	ID_{50} (ng/ml)	ratio
LOVO DX	0	410	1.0	13.0	1.0	460	1.0	12.0	1.0
	1	200	2.0	5.0	2.6	250	1.8	7.5	1.6
	2	75	5.5	4.2	3.1	175	2.6	7.4	1.6
	3	50	8.2	3.7	3.5	120	3.8	7.0	1.7
	6	30	13.7	2.5	5.2	70	6.6	6.5	1.8
	10	15	27.3	2.5	5.2	50	9.4	6.0	2.0
CEM VLB	0	78	1.0	3.2	1.0	100	1.0	3.0	1.0
	1	29	2.7	2.5	1.3	38	2.6	2.5	1.2
	2	18	4.3	2.0	1.6	31	3.2	2.3	1.3
	3	14	5.6	2.0	1.6	22	4.5	2.2	1.4
	6	10	7.8	1.8	1.8	17	5.9	2.0	1.5
	10	5	15.6	1.6	2.0	14	7.1	1.5	2.0

Each number represents the mean of three values. Standard deviation is not represented, but was always 10% or less. The ratios indicate the relative increase of cytotoxicity by increasing concentrations of D-VRP. That increase was much higher for DNR than for IDA, and for DX than for IDX.

drugs (1000 ng/ml) without and with D-VRP (10 μM), and intracellular drug concentration was measured after 1, 2 and 3 h as described. Results are shown in Figure 4 for the LOVO cell system and in Figure 5 for the CEM cell system. In both cell systems, intracellular drug concentration was higher for the parental sensitive line than for the MDR subline. In both cell systems, D-VRP increased intracellular drug concentration only in the MDR subline. The data are summarized in Table 5, showing that the intracellular anthracycline concentration was not modified in the parental, non-MDR cell lines (LOVO 109 and CEM). In contrast, D-VRP increased intracellular anthracycline concentration in the MDR cells (LOVO DX and CEM VLB). The increase was higher in LOVO DX than in CEM VLB, and was higher for the DNR than for IDA, but it was not different for DX and IDX.

Table 3. LOVO cell system: effect of increasing concentration of D-VRP on the RI

	RI (ID_{50} LOVO DX/ID_{50} LOVO 109)			
D-VRP (μM)	DNR	IDA	DX	IDX
0	41.0	5.2	30.6	1.6
1	20.0	2.0	16.7	1.2
2	7.9	1.7	14.6	1.2
3	5.5	1.5	12.0	1.2
6	3.7	1.0	7.0	1.1
10	2.0	1.0	5.5	1.2

Table 4. CEM cell system: effects of increasing concentrations of D-VRP on the RI

	RI (ID_{50} CEM VLB/ID_{50} CEM)			
D-VRP (μM)	DNR	IDA	DX	IDX
0	26.0	2.7	25.0	1.4
1	9.7	2.1	9.5	1.1
2	6.0	1.7	7.7	1.0
3	4.7	1.7	5.5	1.0
6	3.3	1.5	4.2	0.9
10	1.7	1.3	3.5	0.7

Table 5. Effect of D-VRP 10 μM on intracellular drug concentration on MDR (LOVO DX and CEM VLB) and in parental, non-MDR cell lines (LOVO 109 and CEM) exposed for 3 h to the tested drugs at a concentration of 1000 ng/ml

	DNR	IDA	DX	IDX
LOVO DX	4.54	2.17	2.03	1.87
LOVO 109	1.00	1.01	1.00	1.00
CEM VLB	3.81	1.37	1.32	1.33
CEM	0.96	1.08	0.98	1.09

The numbers in the table were obtained by dividing the mean NMFI of the cells with and without D-VRP. A value of 1 indicates no effect of D-VRP on intracellular drug concentration, as it was the case with the parental, non-MDR lines LOVO 109 and CEM. A value greater than 1 indicates that D-VRP increased intracellular drug concentration. Such an increase was higher in LOVO DX cells than in CEM VLB cells, and was higher for DNR than for IDA, but not for DX than for IDX.

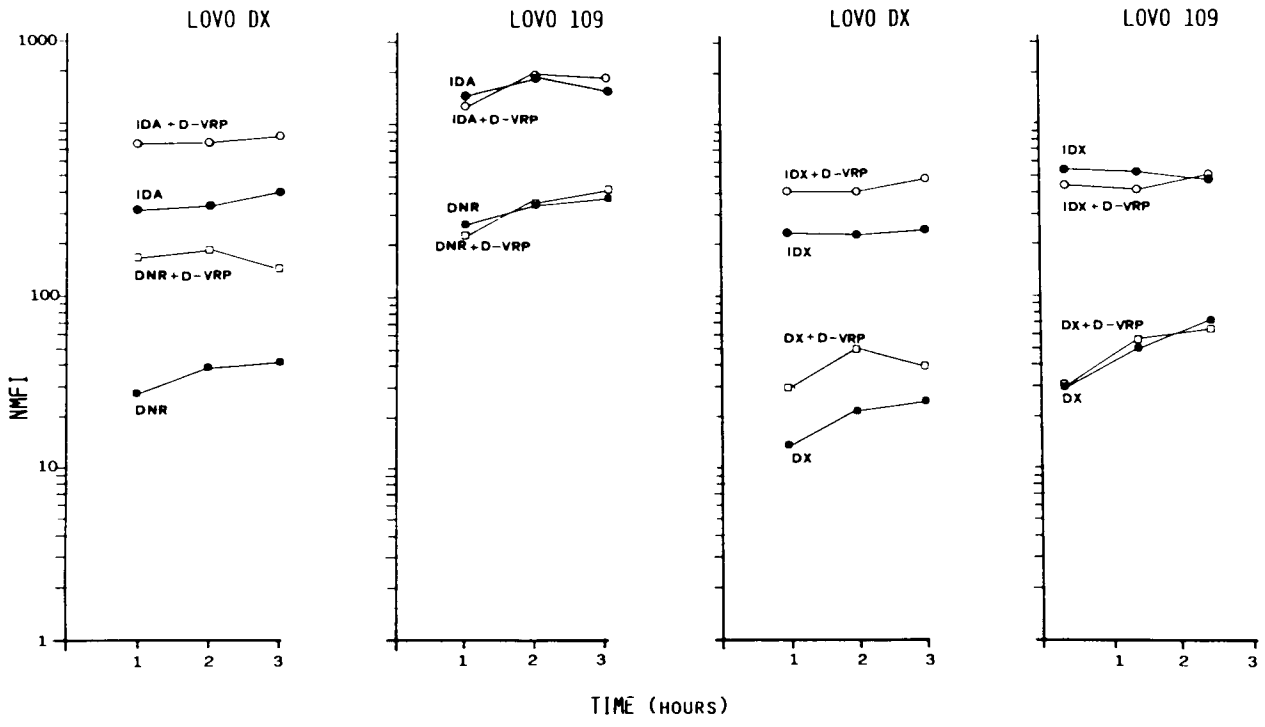

Figure 4. DNR, IDA, DX and IDX intracellular content in the LOVO cell system. Cells were incubated with 1 μg/ml of anthracycline for 1, 2 or 3 h alone or plus D-VRP 10 μM. Each point represents the results of three different experiments. Standard deviation was always 10% or less.

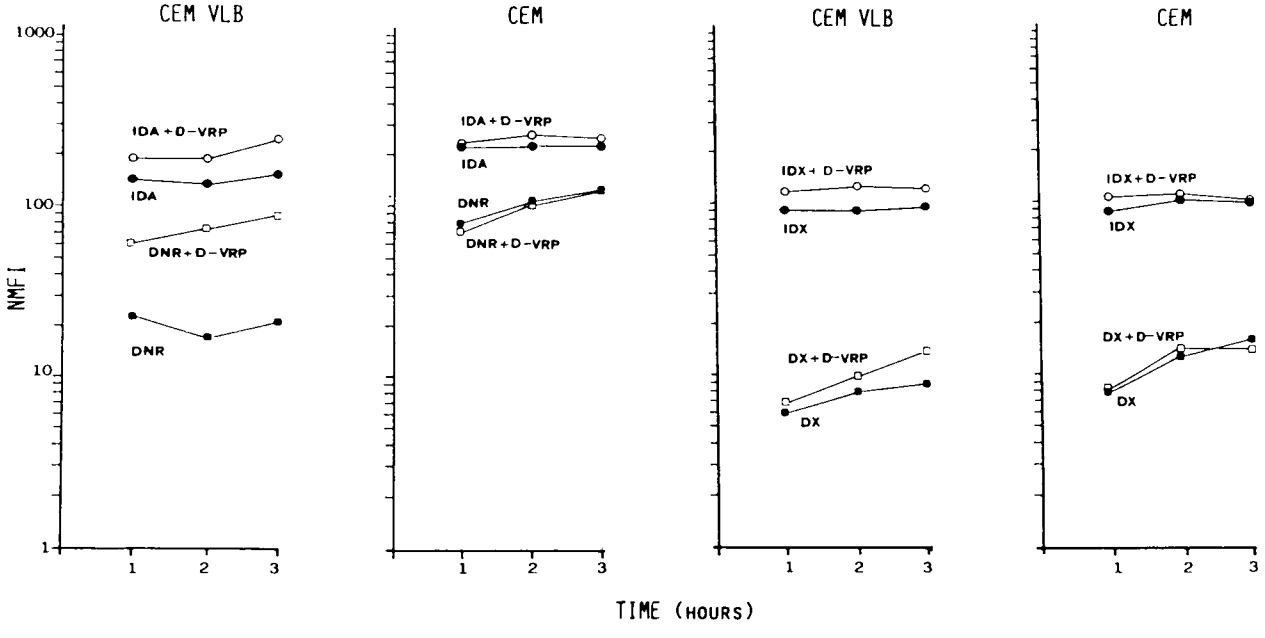

Figure 5. DNR, IDA, DX and IDX intracellular content in the CEM cell system. Cells were incubated with 1 μg/ml of anthracycline for 1, 2 or 3 h alone or plus D-VRP 10 μM. Each point represents the results of three different experiments. Standard deviation was always 10% or less.

Discussion

This study showed that D-VRP at concentrations ranging between 1 and 10 μM affected intracellular anthracycline concentration and anthracycline cytotoxicity in MDR cell lines but not in non-MDR ones. These results were expected, based on prior studies of the racemic mixture (VRP)[9,16] and on several studies of the D-isomer (D-VRP).[35,38] However, it should not be overlooked that prior studies were limited to Vinca alkaloids and tested higher D-VRP concentrations. Our data confirmed that D-VRP is a candidate for the clinical downmodulation of MDR to anthracyclines, since effective reverting could be detected at concentrations of 2–3 μM, which can be safely maintained *in vivo*.[21,23,39] However, we found that downmodulation of MDR by D-VRP could be substantially different depending on the anthracyclines. These differences were summarized in Tables 3 and 4, which showed the potentiation of anthracycline cytotoxicity by D-VRP, at the ID_{50} of each tested drugs. There are three main points. First, the potentiation effect of D-VRP was always better detected with DNR than with DX, since at equal D-VRP concentration, the effect was 2–4 times greater with DNR than with DX. Second, the potentiating effect of D-VRP was always greater with the parent compounds (either DNR or DX) than with the respective lypophylic derivatives (either IDA or IDX). This was expected and fitted with prior results from others,[16,40] and from our laboratory,[32] showing that MDR tumor cells which are resistant to DNR and DX are less resistant to IDA. This point was recently stressed by Berman and McBride[40] who found that at a drug concentration of 1000 ng/ml the cytotoxicity of IDA could not be potentiated by VRP 10 μM. However, since that IDA concentration was more than 100 times higher than the ID_{50} for LOVO DX, CEM VLB and other MDR lines, it was obvious that at such a high IDA concentration any reverting or potentiating effect could not be detected. In this study, we tested much lower IDA concentrations, in the range of its ID_{50}, and we found that a potentiating effect of D-VRP was easily detectable, especially in the LOVO cell system (Table 3). Third, the dose–response relationship was much more important for DNR and for DX than for IDA and IDX. As an example, increasing the D-VRP concentration from 3 to 10 μM potentiated DNR by a factor of about 3, but potentiated IDA only by a factor of about 1.5.

Practical implications of these findings are (i) that D-VRP downmodulates resistance to DNR better than resistance to DX, (ii) that reverting effectively resistance to DNR or to DX with D-VRP alone would require high D-VRP concentrations, close to 10 μM or even higher, and (iii) that reverting resistance to IDA to a level very close to the parental, non-MDR, line can be accomplished at clinically tolerable D-VRP concentrations (2–3 μM). Information on IDX is still insufficient to attempt conclusions or relationships, but it is likely that the interaction between D-VRP and IDX is limited.

The development of anthracycline derivatives and the choice of the anthracycline for treatment will ultimately depend on many factors. Since several independent studies of acute non-lymphocytic leukemia (ANLL) suggested that *mdr-1* gene overexpression is associated with early treatment failure,[41–45] and since anthracyclines are a first-line drug for ANLL, it is important to point out again that although IDA is already less dependent than other anthracyclines of P170-associated resistance,[32,40] residual resistance can be significantly decreased by D-VRP. It is urgent to investigate if the combination of IDA with D-VRP or other resistance modifiers would be more toxic for normal, P170-positive tissues, like hemopoietic stem cells, liver and intestinal mucosa, as well as for myocardial cells.

References

1. Moscow JA, Cowan KH. Multidrug resistance. *J Natl Cancer Inst* 1988; **80**: 14–20.
2. Kaye SB. The multidrug resistance phenotype. *J Cancer* 1988; **58**: 691–4.
3. Nooter K, Herweijer H. Multidrug resistance (*mdr*) genes in human cancer. *Br J Cancer* 1991; **63**: 663–9.
4. Pastan I, Gottesman M. Multiple drug resistance in human cancer. *N Engl J Med* 1987; **316**: 1388–93.
5. Weistein RS, Kuszak JR, Kluskens LF, *et al.* P-glycoprotein in pathology: the multidrug resistance gene family in humans. *Human Pathol* 1990; **21**: 34–48.
6. Boesch D, Gaveriaux C, Jachez B, *et al. In vivo* circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. *Cancer Res* 1991; **51**: 4226–33.
7. Berman E, Adams M, Duigou-Osterndorf R, *et al.* Effect of tamoxifen on cell lines displaying the multidrug-resistant phenotype. *Blood* 1991; **77**: 818–25.
8. Krishan A, Sridhar KS, Davila E, *et al.* Patterns of anthracycline retention modulation in human tumor cells. *Cytometry* 1987; **8**: 306–14.
9. Cass CE, Janowska-Wieczorek A, Lynch MA, *et al.* Effect of duration of exposure to verapamil on vincristine activity against multidrug-resistant human leukemic cell lines. *Cancer Res* 1989; **49**: 5798–5804.
10. Bruno NA, Slate DL. Effect of exposure to calcium entry blockers on doxorubicin accumulation and cytotoxicity in

multidrug-resistant cells. *J Natl Cancer Inst* 1990; **82**: 419–24.
11. Mickisch GH, Kossig J, Keilhauer G, *et al.* Effects of calcium antagonists in multidrug resistant primary human renal cell carcinoma. *Cancer Res* 1990; **50**: 3670–74.
12. Figueredo A, Arnold A, Goodyear M, *et al.* Addition of verapamil and tamoxifen to the initial chemotherapy of small cell lung cancer. *Cancer* 1990; **65**: 1895–902.
13. Tidefelt U, Sundman-Engberg B, Paul C. Effects of verapamil on uptake and in vitro toxicity of anthracyclines in human leukemic blast cells. *Eur J Haematol* 1988; **40**: 385–95.
14. Pennock GD, Dalton WS, Roeske WR, *et al.* Systemic toxic effects associated with high-dose verapamil infusion and chemotherapy administration. *J Natl Cancer Inst* 1991; **83**: 105–10.
15. Cano-Gauci DF, Riordan JR. Action of calcium antagonists on multidrug resistant cells. Specific cytotoxicity independent of increased cancer drug accumulation. *Biochem Pharmacol* 1987; **36**: 2115–23.
16. Muller MR, Lennartz K, Boogen C, *et al.* Cytotoxicity of adriamycin, idarubicin, and vincristine in acute myeloid leukemia: chemosensitization by verapamil in relation to P-glycoprotein expression. *Ann Hematol* 1992; **65**: 206–12.
17. Solary E, Bidan JM, Calvo F, *et al.* P-glycoprotein expression and *in vitro* reversion of doxorubicin resistance by verapamil in clinical specimens from acute leukaemia and myeloma. *Leukemia* 1991; **5**: 592–7.
18. Salmon SE, Dalton WS, Grogan TM, *et al.* Multidrug-resistance myeloma: laboratory and clinical effects of verapamil as a chemosensitizer. *Blood* 1991; **78**: 44–50.
19. Durie BGM, Dalton WS. Reversal of drug-resistance in multiple myeloma with verapamil. *Br J Haematol* 1988; **68**: 203–6.
20. Dalton WS, Grogan TM, Meltzer PS, *et al.* Drug-resistance in multiple myeloma and non-Hodgkin's lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. *J Clin Oncol* 1989; **7**: 415–24.
21. Eichelbaum M, Mikus G, Vogelgesang B. Pharmacokinetics of (+)-, (−)- and (±)-verapamil after intravenous administration. *Br J Clin Pharmacol* 1984; **17**: 453–8.
22. Bright JM, Buss DD. Effects of verapamil on chronic doxorubicin-induced cardiotoxicity in dogs. *J Natl Cancer Inst* 1990; **82**: 963–4.
23. Echizen H, Brecht T, Niedergesass S, *et al.* The effect of dextro-, levo-, and racemic verapamil on atrioventricular conduction in humans. *Am Heart J* 1985; **109**: 210–7.
24. Vayuvegula B, Slater L, Meador J, *et al.* Correction of altered plasma membrane potentials. *Cancer Chemother Pharmacol* 1988; **22**: 163–8.
25. Naito M, Tsuruo T. Competitive inhibition by verapamil of ATP-dependent high affinity vincristine binding to the plasma membrane of multidrug-resistant K562 cells without calcium ion involvement. *Cancer Res* 1989; **49**: 1452–5.
26. Drewinko B, Romsdahl MM, Yang LY, *et al.* Establishment of a human carcinoembryonic antigen-producing colon adenocarcinoma cell line. *Cancer Res* 1976; **36**: 467–75.
27. Grandi M, Geroni C, Giuliani FC. Isolation and characterization of a human colon adenocarcinoma cell line resistant to doxorubicin. *Br J Cancer* 1986; **54**: 515–8.
28. Foley GE, Lazarus H, Farber S, *et al.* Continuous culture of human lymphoblasts from peripheral blood of child with acute leukemia. *Cancer* 1965; **18**: 522–9.
29. Beck WT, Mueller TJ, Tanzer LR. Altered surface membrane glycoprotein in vinca alkaloid-resistant human leukemic lymphoblasts. *Cancer Res* 1979; **39**: 2070–6.
30. Ballinari D, Radice P, Grandi M, *et al.* MDR amplification and karyotypic analysis of anthracycline-resistant human tumor cell lines. *Cancer Commun* 1988; **3**: 69–74.
31. Shen DW, Fojo A, Chin LE, *et al.* Human multidrug-resistant cell lines: increased *mdr-1* expression can precede gene amplification. *Science* 1986; **232**: 633–45.
32. Michieli M, Michelutti A, Damiani D, *et al.* A comparative analysis of the sensitivity of multidrug resistant (MDR) and non-MDR cells to different anthracycline derivatives. *Leukemia and Lymphoma* 1993; in press.
33. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; **65**: 55–63.
34. Luk CK, Tannock IF. Flow cytometric analysis of doxorubicin accumulation in cells from human and rodent cell lines. *J Natl Cancer Inst* 1989; **81**: 55–9.
35. Mickisch GH, Merlino GT, Galski H, *et al.* Transgenic mice that express the human multidrug-resistance gene in bone marrow enable a rapid identification of agents that reverse drug resistance. *Proc Natl Acad Sci USA* 1991; **88**: 547–51.
36. Mickisch GH, Merlino GT, Aiken PM, *et al.* New potent verapamil derivatives that reverse multidrug resistance in human renal carcinoma cells and in transgenic mice expressing the human mdr1 gene. *J Urol* 1991; **146**: 447–53.
37. Ellason JF, Ramuz H, Kaufmann F. Human multi-drug-resistant cancer cells exhibit a high degree of selectivity for stereoisomers of verapamil and quinidine. *Int J Cancer* 1990; **46**: 113–7.
38. Gruber A, Peterson C, Reizenstein P. D-verapamil and L-verapamil are equally effective in increasing vincristine accumulation in leukemic cells *in vitro*. *Int J Cancer* 1988; **41**: 224–6.
39. Bissett D, Kerr DJ, Cassidy J, *et al.* Phase I and pharmacokinetic study of D-verapamil and doxorubicin. *Br J Cancer* 1991; **64**: 1168–71.
40. Berman E, McBride M. Comparative cellular pharmacology of daunorubicin and idarubicin in human multidrug-resistant leukemia cells. *Blood* 1992; **79**: 3267–3273.
41. Campos L, Guyotat D, Archimbaud E, *et al.* Clinical significance of multidrug resistance P-glycoprotein expression on acute nonlymphoblastic leukemia cells at diagnosis. *Blood* 1992; **79**: 473–6.
42. Michieli M, Damiani D, Geromin A, *et al.* Overexpression the multidrug resistance-associated P170-glycoprotein in acute non-lymphocytic leukemia. *Eur J Haematol* 1992; **48**: 87–92.
43. Sato H, Preisler H, Day R, *et al.* MDR1 transcript levels as an indication of resistant disease in acute myelogenous leukaemia. *Br J Haematol* 1990; **75**: 340–5.
44. Marie JP, Zittoun R, Sikic BI. Multidrug resistance (*mdr-1*) gene expression in adult acute leukemias: correlations with treatment outcome and *in vitro* drug sensitivity. *Blood* 1991; **78**: 586–592.
45. Pirker R, Wallner J, Geissler K, *et al.* MDR1 gene expression and treatment outcome in acute myeloid leukemia. *J Natl Cancer Inst* 1991; **83**: 708–12.

(Received 21 January 1993; accepted 3 February 1993)