DIFFERENTIAL SENSITIVITY OF MULTI-DRUG-RESISTANT AND -SENSITIVE CELLS TO RESISTANCE-MODIFYING AGENTS AND THE RELATION WITH REVERSAL OF ANTHRACYCLINE RESISTANCE

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Calcium channel blockers, calmodulin inhibitors, and some other classes of non-related compounds reverse multi-drug resistance. In the present study, we found that several resistance modifiers are more toxic for MDR cells than for the corresponding sensitive parent cells, whereas others show the opposite effect. Several calcium channel blockers including bepridil, diltiazem, nifedipine and verapamil, as well as the calmodulin inhibitor trifluoperazine, were more toxic for several MDR cell lines than for the parent cell lines. In contrast, cross-resistance for cyclosporin A and the verapamil analogue Ro 11-2933/001 was observed in the MDR/sensitive cell couple $CH^R C_5/AUXB_1$, probably due to a concentration-dependent stimulation of cell growth in the range of 0–4 μM cyclosporin A and of 1–4 μM Ro 11-2933/001. In partially revertant $CH^R C_5$ cells, growth inhibition by Ro 11-2933/001 at concentrations below 1 μM , as seen in $CH^R C_5$ cells, changed into growth stimulation, and the collateral sensitivity to verapamil and bepridil disappeared almost completely. In the MDR cells $CH^R C_5$, 2780^{AD} and DC3F/DMXX, cross-resistance to another calcium channel blocking agent, Ro 11-1781/001 (tiapamil), was observed as well. This compound showed exceptional behavior: it induced marked potentiation of Dx cytotoxicity as well as stimulation of Dx accumulation in $AUXB_1$ cells, even at low tiapamil concentrations, but not in the $CH^R C_5$ cells, even at high concentrations. It is concluded that resistance modifiers can selectively influence growth of MDR cells via more than one process, and resulting in either strong growth inhibition in MDR cells relative to the effect on sensitive cells or in growth stimulation.

Many compounds, including calcium entry blockers and calmodulin inhibitors, are able to reverse resistance to anti-cancer drugs such as anthracyclines and Vinca alkaloids in multi-drug-resistant cells (Helson, 1984; Kessel, 1986). This reversal is considered to occur via enhancement of drug accumulation, probably by inhibiting drug efflux (Helson, 1984; Kessel, 1986) or via a change in intracellular drug distribution (Schuurhuis *et al.*, 1989; Keizer *et al.*, 1989). In earlier studies on the potency of the calcium entry blocker bepridil (Bp) to reverse doxorubicin (Dx) and daunomycin (DM) resistance in MDR cells, performed with verapamil (Vp) as reference compound (Schuurhuis *et al.*, 1987; Broxterman *et al.*, 1987a), we found that both calcium entry blockers, but especially Bp, could be selectively toxic to MDR cells (Schuurhuis *et al.*, 1987). This has also been noted by other investigators (Warr *et al.*, 1986, 1988; Cano-Gauci and Riordan, 1987; Twentyman *et al.*, 1987). Since such specific toxicity might be of potential use in cancer chemotherapy and might also shed light on the mechanism(s) underlying multi-drug resistance, we decided to study this phenomenon in more detail using other resistance-modifying agents (RMAs) and other MDR/sensitive cell line couples. The effects of Bp, Vp, trifluoperazine (TFP), diltiazem (Dilt), nifedipine (Nif), tamoxifen (TMF), and cyclosporin A, all known to be able to reverse MDR (Schuurhuis *et al.*, 1987; Broxterman *et al.*, 1987a; Tsuruo *et al.*, 1983; Ganapathi *et al.*, 1985; Slater *et al.*, 1986; Ramu *et al.*, 1984), were studied in Chinese hamster ovarian and lung cells as well as in human ovarian cancer, breast cancer and squamous lung cancer cells. In addition, 2 Vp analogues, Ro 11-1781/001 and Ro 11-2933/001, previously studied in P388 mouse leukemia

cells (Kessel and Wilberding, 1985), were evaluated with respect to their ability to stimulate Dx accumulation, potentiate Dx cytotoxicity, and affect cell growth in MDR cells in a different way from that observed in sensitive cells.

MATERIAL AND METHODS

Drugs

Bepridil monohydrochloride monohydrate was a gift of Organon International (Oss, The Netherlands); Ro 11-2933/001 (Ro) and Ro 11-1781/001 (tiapamil) were provided by Hoffmann-La Roche (Basel, Switzerland). Verapamil · HCl, diltiazem · HCl, trifluoperazine, nifedipine, and tamoxifen citrate were obtained from Sigma (St. Louis, MO). Cyclosporin A (Sandimmune®) was provided by Sandoz (Basel, Switzerland). Doxorubicin was obtained from Roger Bellon (Neuilly sur Seine, France), [¹⁴C] doxorubicin (53.3 $\mu Ci/\mu Mol$) from Amersham (Little Chalfont, UK) as well as [¹⁴C] daunomycin (50 $\mu Ci/\mu Mol$).

Stock solutions were prepared in 0.9% NaCl (Dx, DM, Vp, TFP, Dilt) or in DMSO (Ro, Nif). Bp was obtained as a 4 mg/ml solution in saline containing 48.4 mg/ml glucose, tiapamil as a 10 mg/ml solution in saline and cyclosporin A as a 10 mg/ml solution in Cremophor EL plus ethanol.

Cells and cell culture

The Chinese hamster ovarian cell line $AUXB_1$ and its colchicine-resistant subline $CH^R C_5$ were kindly supplied by Dr. V. Ling (Ontario Cancer Institute, Toronto, Canada). The human ovarian cancer cell line A2780 and its Dx-resistant subline 2780^{AD} was a gift of Dr. R.F. Ozols (NCI, Bethesda, MD); the Chinese hamster lung cell line DC3F and its DM-resistant counterpart were supplied by Dr. J.L. Biedler (Sloan-Kettering Cancer Center, New York) and the human breast cancer cell line MCF-7 and its Dx-resistant subline MCF-7/Adr^R by Dr. K. Cowan (NCI, Bethesda, MD). The human ovarian cancer cell line H134 was established as described elsewhere (Broxterman *et al.*, 1987b). The human squamous lung cell line SW-1573 was established by Dr. A. Leibowitz (Scott and White Clinic, Temple, TX); the Dx-resistant sublines SW-1573/1R500 (Keizer *et al.*, 1989) and H134^{AD} were obtained by growing sensitive cells in the presence of Dx in stepwise-increased concentrations.

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Abbreviations: Bp, bepridil; Dilt, diltiazem; DMF, dose-modifying factor; IC₅₀ without RMA/IC₅₀ with RMA; DM, daunomycin; Dx, doxorubicin; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid; IC₅₀, drug concentration resulting in 50% inhibition of cell growth; MDR, multi-drug-resistant/resistance; Nif, nifedipine; RMA, resistance-modifying agent; Ro, Ro 11-2933/001; TFP, trifluoperazine; TMF, tamoxifen; Tp, tiapamil; Vp, verapamil.

The cells were cultured in plastic flasks (Greiner, Nürtingen, FRG) in a humidified atmosphere at 37°C, 5% CO₂. The human ovarian-, breast- and lung-cancer cells were maintained in Dulbecco's modified Eagle's medium containing glutamine (Gibco, Paisley, Scotland), as well as 20 mM HEPES. The Chinese hamster cell lines were cultured in MEM alpha medium and the Chinese hamster lung cell lines in MEM medium (Gibco). All culture media were supplemented with 10% fetal bovine serum (Flow, Irvine, Scotland). The resistant cells were cultured in the presence of the appropriate drug (10 µg/ml colchicine for CH^RC₅, 2 µM Dx for 2780^{AD}, 0.5 µM Dx for SW-1573/1R500, 2 µM Dx for H134^{AD}, 10 µM Dx for MCF-7/Adr^R, and 17.8 µM DM for DC3F/DMXX).

All resistant cell lines exhibited cross-resistance to other anthracyclines, VP-16, and Vinca alkaloids. Resistance indices (IC₅₀ MDR cells/IC₅₀ sensitive cells) for Dx were: 72 (2,780 cells); 155 (human lung-cancer cells); 348 (Chinese hamster ovarian cells) 1,600 (human breast-cancer cells) and for DM: 2,500 (DC3F cells), and 225 (H134 cells) as determined in a 2-hr or continuous drug exposure assay (see "Drug treatment" below). A partially revertant cell line of CH^RC₅ was obtained after culturing CH^RC₅ cells for 1 year in the absence of colchicine. The level of Dx resistance had decreased from 348 to 112.

Drug treatment

Adherent cells were detached with a solution of trypsin (0.025 mg/l) containing EDTA (2.5 mg/ml) in Ca⁺⁺- and Mg⁺⁺-free Hanks' buffered salt solution and plated on 6-well tissue culture plates (Costar, Cambridge, MA) at a density of 3 to 6 × 10⁴ cells per well. After 18 to 24 hr log-phase cells were incubated with the appropriate drugs at 37°C. For assessment of toxicity of resistance-modifying agents (RMAs), the cells were exposed to the compounds for 48 to 72 hr, after which the cell counts were performed. Dx cytotoxicity and the effects of RMAs on it were determined in a 2-hr incubation assay (Schuurhuis *et al.*, 1987). After combined Dx-RMA treatment for 2 hr, the cells were incubated for another 2 hr in the presence of the RMA only and finally in drug-free medium for 48 to 72 hr. In some experiments (see "Results") the RMA was continuously present after combined Dx-RMA treatment. After that, cells were counted with a Coulter Counter (Sigmex microcell counter CC-110), and cell numbers were corrected for the number present when the drug was added.

Drug accumulation

Drug accumulation studies were carried out as described elsewhere (Schuurhuis *et al.*, 1987). In short, trypsinized cells were washed and resuspended in growth medium containing 20 mM HEPES but without NaHCO₃. About 0.5 × 10⁶ cells were incubated at 37°C in a total volume of 550 µl containing 2 µM [14-¹⁴C] Dx plus the desired concentration of RMA. After 90 min, accumulation was arrested with ice-cold PBS. After another wash, the cells were transferred to liquid scintillation fluid Opti-fluor (Packard, Groningen, The Netherlands) and counted. Values at 90 min were corrected for the amounts of cell-associated Dx present at time zero at 0°C.

ATP measurements

Cells (10⁶ per 1 ml sample) were incubated for 10 min at 37°C in growth medium without glucose but containing 10% fetal calf serum and 10 mM sodium azide. ATP/ADP measurements were carried out with the use of an ion-exchange high-performance liquid chromatography system (Broxterman *et al.*, 1988).

RESULTS

Potentiation of Dx cytotoxicity in MDR and sensitive cells

These results are summarized in Table I and Figure 1. In

TABLE I - RMA-INDUCED POTENTIATION OF DX CYTOTOXICITY IN MDR AND SENSITIVE CELLS

RMA	Concentration (µM)	DMF ¹			
		AUXB ₁	CH ^R C ₅	A2780	2780 ^{AD}
Bp	2	1.4 ²	9	1.0	2.8
	4	1.4	67	1.0	8.7
	8	— ³	136	—	—
Vp	2	1.8	25	1.0	3.7
	4	2.0	87	1.0	8.4
	8	—	200	—	—
Ro	1	—	100	—	15
	2	—	294	—	18
	4	2.2	313	1.1	19
Tp	32	1.8	1.0	—	—
	64	—	1.0	1.1	1.3
	128	1.9	1.3	—	—
	256	—	—	1.1	5.0

¹DMF, dose-modifying factor as determined in a 2-hr incubation assay (for details see "Material and Methods"). ²Values are means of at least 2 experiments (SD < 20%). ³—, Not determined.

general, the order of potentiation of both parameters by the resistance-modifying agents (RMAs) is similar for both types of resistant cell: Ro > Vp > Bp > Tp. All 4 RMAs showed effects on AUXB₁ but not on A2780 cells (Table I and Fig. 1).

The compound Tp showed exceptional behavior. It was not active in potentiating Dx sensitivity (Table I) or stimulating Dx accumulation (Fig. 1) in CH^RC₅ and 2780^{AD} cells at concentrations up to 64 µM and, like the other RMAs, it had no effect on A2780 cells (Table I, Fig. 1). However, it potentiated Dx cytotoxicity in AUXB₁ cells at relatively low concentrations, which did not affect Dx cytotoxicity in CH^RC₅ (Table I). Dx accumulation in AUXB₁ cells was stimulated as well (Fig. 1a). Maximal effects of Dx cytotoxicity were found when the AUXB₁ cells were incubated for prolonged times with the RMA after combined Dx-RMA treatment (Fig. 2). Maximal effects of Tp were the same as those found for Vp. With this treatment, too, there was no effect on CH^RC₅ cells except at very high concentrations (Fig. 2). Under the same conditions Ro was very potent in CH^RC₅ cells (Fig. 2).

Toxicity of resistance modifiers in MDR and sensitive cells

Previous studies with Vp and Bp had shown that these compounds were more toxic to MDR cell lines than to their sensitive counterparts (Schuurhuis *et al.*, 1987). Furthermore, when used in concentrations that unequivocally potentiated Dx cytotoxicity, the RMAs were found to be subtoxic to toxic by themselves (Schuurhuis *et al.*, 1987). To find out whether increased sensitivity of MDR cells to RMAs is a general phenomenon and whether it corresponds to the ability of RMAs to potentiate anthracycline cytotoxicity in MDR cells selectively, we studied several RMAs in a number of MDR and sensitive cell lines. Figure 3 shows the effects of Bp, Vp, and Tp on growth of Chinese hamster ovarian (Fig. 3a), Chinese hamster lung (Fig. 3b), and human ovarian carcinoma (Fig. 3c) cells. Generally, Bp and Vp were more toxic for all 3 MDR cells than for the sensitive cells, but the effect was most pronounced in the hamster cell lines, *e.g.* Vp in the CHO cells (factor 34). A similar collateral sensitivity to Bp was also found in several other human MDR cells including the squamous lung-cancer cell line SW-1573/1R500 (factor 1.3 at IC₅₀), the ovarian-cancer cell line H134^{AD} (factor 1.5) as well as the human breast-cancer cell line MCF-7/Adr^R (factor 1.5). Tp, on the other hand, was more toxic for all 3 sensitive cells which, at least in one pair of cell lines (2780^{AD}/A2780), did not parallel the ability of Tp to affect Dx cytotoxicity (compare Table I and Fig. 3c). Further, Tp was the only compound that potentiated

FIGURE 1 – RMA-induced stimulation of Dx accumulation in MDR and sensitive cells. Dx accumulation was determined after 90 min of incubation at 37°C with 2 μM Dx plus or minus RMAs. (a) AUXB₁ and CH^RC₅; (b) A2780 and 2780^{AD}. Open symbols: MDR cells; closed symbols: sensitive cells. ○—●, Bp; □—■, Vp; △—▲, Tp; ▽—▼, Ro. Symbols represent mean ± SE of 3–8 independent experiments, each performed at least in duplicate.

Dx cytotoxicity in AUXB₁ but not in CH^RC₅ at doses far below toxic levels (compare Figs. 2 and 3a).

Figure 4 shows the effects of Ro on the 3 cell lines. In the Chinese hamster lung cell lines Ro was more toxic for the MDR cell line at all concentrations (Fig. 4b) and in the human ovarian-cancer cell line a slight effect was seen at concentrations above 4 μM (Fig. 4c). Remarkably, for the Chinese hamster ovarian cell line, Ro was more toxic for the MDR cell lines at concentrations below 3 μM, but less toxic at concentrations higher than 3 μM, as a result of a dose-dependent cell growth stimulation by Ro at concentrations between 1 and 8 μM (Fig. 4a). A similar, though less pronounced, effect was seen in the MDR Chinese hamster lung cell line (Fig. 4b). This effect did not parallel Ro effects on Dx cytotoxicity, since in CH^RC₅ a normal dose-dependent increase in the potentiation of Dx cytotoxicity was found in the concentration range 1–4 μM in short-term incubations with Ro plus Dx (Table I) as well as in continuous incubations with Ro plus Dx (data not shown).

Another exception to the pattern of collateral sensitivity of MDR cells to effective RMAs is shown in Figure 5: CH^RC₅ cells were cross-resistant to cyclosporin A (Fig. 5a), a tendency also seen for 2780^{AD} cells at concentrations <1 μM or >8 μM (Fig. 5b). This is, at least in CH^RC₅ cells, probably due to a cyclosporin-A-induced stimulation of cell growth already occurring at low concentrations (0–4 μM) and, like Ro in CH^RC₅ (Fig. 4a), is seen only in resistant cells.

FIGURE 2 – RMA-induced potentiation of Dx cytotoxicity in AUXB₁ and CH^RC₅. DMFs for Bp, Vp, Ro or Tp were determined as described in "Material and Methods" except that the initial 2-hr incubation period with the Dx-RMA combination was followed by continuous incubation (48 to 72 hr) with the RMA alone. No data are shown for Bp and Vp in CH^RC₅ (except for 1 μM Bp) because these compounds are too toxic in continuous incubation. Open symbols: MDR cells; closed symbols: sensitive cells. ○—●, Bp; □—■, Vp; △—▲, Tp; ▽—▼, Ro. Symbols represent mean ± SE of 2–4 independent experiments except in the case of single experiments (indicated by an asterisk).

Incubation with resistance modifiers such as Vp induces energy depletion selectively in MDR cells under appropriate conditions (Broxterman *et al.*, 1988). To find out whether such an effect is correlated with the ability of RMAs to inhibit or stimulate cell growth in MDR cells selectively, we assessed the effects of Ro on energy consumption in CH^RC₅ cells in the concentration range of interest. Figure 6 shows that the concentration dependence of Ro-induced energy depletion is similar to that found for Vp (Broxterman *et al.*, 1988) and does not reflect the anomalous effects of the compound on cell growth (Fig. 4).

Reduction of resistance occurring in MDR cells cultured for prolonged periods in drug-free medium was accompanied by a decrease in sensitivity to RMAs, as illustrated by the findings for CH^RC₅ (Fig. 7). Remarkably, the selective toxicity of Bp and Vp for the MDR cell line CH^RC₅ disappeared substantially or completely (Fig. 7a,b) despite the relatively small decrease in the level of Dx resistance (348 to 112). The same effect was found for the toxic component in the 3-phasic curve of Ro in CH^RC₅. Drug-free incubation of the cells led to a slight growth stimulation over the whole concentration range of 0–8 μM Ro (Fig. 7c). Cyclosporin-A-induced growth stimulation as seen in CH^RC₅ cells was still present in the revertant cell line (Fig. 7d). This cell line, unlike the parental cell line AUXB₁, still contained large amounts of P-glycoprotein (not shown).

Comparable differential toxicity for MDR and sensitive cells was also found with other RMAs. Figure 8 shows the toxic effects of the calcium channel blockers Nif and Dilt, the calmodulin inhibitor TFP, as well as TMF, on MDR and sensitive cells. Dilt, Nif, TFP or TMF in Chinese hamster ovarian cells (Fig. 8a) and Dilt and Nif in the human ovarian-cancer cells (Fig. 8b) showed the same pattern of sensitivity as described for *e.g.*, Bp, but only a marginal difference was found for TFP at concentrations <8 μM in 2780 cells. Due to plateau formation in the dose-response curve of Dilt in CH^RC₅ cells, the differential effect was strongly dependent on the RMA concentration (Fig. 8a); for example, the DMF at IC₆₀ is 14 whereas the DMF at IC₅₀ is only 1.7. A similar though less pronounced effect was seen in 2780 cells (Fig. 8b).

FIGURE 3 – Toxicity of Tp, Vp, and Bp for MDR and sensitive cells. (a) $CHRC_5$ and $AUXB_1$; (b) $DC3F/DMXX$ and $DC3F$; (c) 2780^{AD} and $A2780$. Log-phase cells were incubated for 48 to 72 hr at $37^\circ C$ in the presence of the indicated concentrations of RMAs (for details, see "Material and Methods"). Dashed lines (closed symbols) refer to the MDR cells, solid lines (open symbols) to the sensitive cells. $\bigcirc$ — $\bigcirc$, Tp; ∇ — ∇ , Vp; $\triangle$ — $\triangle$, Bp. Symbols represent mean $\pm$ SE of 2–6 independent experiments.

FIGURE 4 – Toxicity of Ro 11-2933/001 for MDR and sensitive cells. (a) $CHRC_5$ and $AUXB_1$; (b) $DC3F/DMXX$ and $DC3F$; (c) 2780^{AD} and $A2780$. Cells were treated as described for Figure 3. $\bigcirc$ — $\bigcirc$, sensitive cells; $\bullet$ — $\bullet$, MDR cells. Symbols represent mean $\pm$ SE of 2–4 independent experiments. The slight stimulation of cell growth seen in $DC3F/DMXX$ at $2 \mu M$ Ro was observed in all individual dose-response experiments.

FIGURE 5 – Cyclosporin A toxicity on MDR and sensitive cells. (a) $CHRC_5$ and $AUXB_1$; (b) 2780^{AD} and $A2780$. Cells were treated as described for Figure 3. $\bigcirc$ — $\bigcirc$, sensitive cells; $\bullet$ — $\bullet$, MDR cells. Symbols represent mean $\pm$ SE of 2–5 independent experiments.

DISCUSSION

In this study the ability of several drugs to potentiate Dx sensitivity in MDR and sensitive cells was compared, as well as the ability to affect cell growth of MDR cells in a way different from that seen in parent cells. Of the 4 compounds

FIGURE 6 – Effect of Ro on cellular ATP/ADP ratios in $CHRC_5$ cells. Cells were incubated for 10 min at $37^\circ C$, as described in "Material and Methods". Results are expressed as percentages of the control ATP/ADP ratio measured in sodium-azide-containing medium after 10 min at $37^\circ C$, which is 0.77. Values are mean $\pm$ SD of 2 independent experiments, each performed at least in duplicate.

tested, Ro was the most active (on a medium concentration basis) in reversing Dx resistance in the Chinese hamster ovarian cell line $CHRC_5$ and the human ovarian-cancer cell line 2780^{AD} . These results are in agreement with those reported for P388/Dx (Kessel and Wilberding, 1985) and with a recent report on 2780 cells (Radel *et al.*, 1988).

FIGURE 7 – RMA toxicity for the CHRC₅-revertant cell line. Cells were treated as described for Figure 3, with Bp (6A), Vp (6B), Ro (6C), or cyclosporin A (6D). For comparison, the results obtained with the parent CHRC₅ and AUXB₁ cells, as shown in Figs. 3–5, are also indicated (dashed lines for CHRC₅ and dotted lines for AUXB₁). Symbols represent mean ± SE of 2–3 independent experiments.

As found earlier for P388/Dx cells (Kessel and Wilberding, 1985), the calcium antagonist tiapamil (Haeusler *et al.*, 1982) was not effective in CHRC₅ and 2780^{AD} cells at low concentrations. Tp was, however, the only one of the compounds under study that was active at low concentrations in potentiating drug sensitivity and stimulating drug accumulation in parental cells (AUXB₁), but not in high-level MDR cells (CHRC₅). Moreover, in contrast to the findings for the other resistance modifiers, potentiation occurred at concentrations far below the toxic concentration of the compound. Although the mechanism responsible for this reversed selectivity is not known, this observation might be helpful in the identification of the mechanism(s) by which RMAs act on drug sensitivity.

We have previously reported that Bp and Vp inhibit cell growth of MDR cells at lower concentrations than are required for sensitive cells (Schuurhuis *et al.*, 1987). In the present study these and other RMAs were studied in Chinese hamster and human cell lines and several new aspects of this differential toxicity were found. The most pronounced effects were seen in Chinese hamster cells; less pronounced but still significant effects were observed in human cells.

No correlation was found between the toxicity of the RMA and the ability to reverse Dx resistance, *e.g.*, Vp (at concentrations ≥ 4 μM) was as active as Bp in reversing Dx resistance in 2780^{AD} cells (Schuurhuis *et al.*, 1987) or in stimulating Dx accumulation in 2780^{AD} cells, but was less toxic for the cells than Bp. Ro at concentrations of 0–4 μM was also superior to Bp in reversing Dx resistance or stimulating Dx accumulation

in 2780^{AD} cells, but the toxicity of both compounds was about the same. Furthermore, Tp was more toxic for sensitive human ovarian-cancer cells, although at slightly toxic concentrations it showed the normal selectivity of RMAs for the human ovarian-cancer MDR cells with respect to the potentiation of Dx cytotoxicity. Lastly, Ro showed a dose-dependent cell-growth inhibition or stimulation in CHRC₅ cells, whereas there was a normal dose-dependent potentiation of Dx cytotoxicity.

Others have also found differential sensitivity of MDR and sensitive cells to RMAs, in particular Vp (Warr *et al.*, 1986, 1988; Cano-Gauci and Riordan, 1987; Twentyman *et al.*, 1987; Reeve *et al.*, 1989), but no concentration-dependent effects were reported which result either in collateral sensitivity or in cross-resistance for the same compounds.

It remains an open question why most RMAs inhibit tumor-cell growth preferentially in MDR cells. It is unlikely that differences in Ca⁺⁺ fluxes over the plasma membrane or differences in Ca⁺⁺ accumulation play a role, since these processes are not affected by *e.g.*, Vp in MDR cells (Warr *et al.*, 1986, 1988; Cano-Gauci and Riordan, 1987; Kessel and Wilberding, 1984). The cellular amounts of RMAs do not seem to play a decisive role either, since Vp accumulation is lower in 2780^{AD} cells than in A2780 cells (Broxterman *et al.*, 1988). Similar findings have been made for CHO cells (Cano-Gauci and Riordan, 1987) and human myelogenous leukemia cells (Yusa and Tsuruo, 1989). Vp might affect intracellular targets that are important for cell growth. In this respect, several MDR cells, including CHRC₅, show increased expression

FIGURE 8 – Toxicity of Dilt, Nif, TFP, and TMF for MDR and sensitive cells. (a) $CH^R C_5$ and $AUXB_1$; (b) 2780^{AD} and $A2780$. Cells were treated as described for Figure 3. Dashed lines (closed symbols) refer to MDR cells, solid lines (open symbols) to the sensitive cells. ○—, Dilt; □—, Nif; △—, TFP; ▽—, TMF. Symbols represent mean $\pm$ SE of 3–4 independent experiments (TMF: 2 experiments).

of the low-molecular-weight calcium binding cytosolic protein sorcin (V19) (Meyers and Biedler, 1981; Meyers *et al.*, 1985; Van der Bliek *et al.*, 1986; Hamada *et al.*, 1988). However, the Chinese hamster lung MDR cell line DC3F/DMXX, which does not show either sorcin gene amplification (De Bruijn *et al.*, 1986) or increased protein synthesis (Meyers *et al.*, 1987), is nevertheless very sensitive to RMAs. This makes a direct involvement of sorcin in hypersensitivity of MDR cells towards RMAs unlikely.

The existence of 3 phases in RMA-induced growth perturbation, which was observed most distinctly for Ro in $CH^R C_5$, suggests that in MDR cells at least 2 cellular targets that are involved in cell proliferation are more susceptible to and have different affinities for RMAs in MDR cells. Such changes in targets during development of resistance, and thus changes in the relative expression of the 3 phases, would then depend on the interaction of a particular RMA with one or more of the targets and on the cell line used (this study) or on the cell culture conditions, *e.g.*, the selective toxicity of RMAs for $CH^R C_5$ cells disappeared when colchicine, the selecting drug, was left out of the medium for a prolonged time. Such a hypothesis would account for collateral sensitivity for most of the RMAs, cross-resistance to cyclosporin A, as observed for CHO cells (this study) or to Vp, as observed in mouse MDR cells (Reeve *et al.*, 1989), as well as plateau formation in the dose-response curves in MDR cells (*e.g.*, Dilt in $CH^R C_5$ or 2780^{AD}), a phenomenon also reported by Warr *et al.* (1988). It would also explain why dose-dependent growth inhibition by Ro, as seen at low concentrations in $CH^R C_5$ cells, can change into growth stimulation, and thereby into cross-resistance towards Ro, in drug-revertant $CH^R C_5$ cells at the same Ro concentrations.

Another explanation for collateral sensitivity towards RMAs would be that binding of RMAs to P-glycoprotein (Bradley *et al.*, 1988), which is overexpressed in the cell lines used in this study (Kartner *et al.*, 1985; Scheper *et al.*, 1988), somehow affects cell growth. This possibility is strengthened by the observation that transfection experiments with complementary DNA, encoding for P-glycoprotein, resulted in MDR variants which exhibited collateral sensitivity to Vp (Croop *et al.*, 1987). We have shown that RMAs are able to decrease cellular

ATP levels under certain conditions, probably because these drugs are pumped out actively via P-glycoprotein (Broxterman *et al.*, 1988). However, although the collateral sensitivity might be explained by the presence of P-glycoprotein, it seems unlikely that an increased energy consumption as such would explain the whole complex pattern of growth-inhibitory and stimulatory properties of RMAs.

Clinical implications of this study may include intrinsic anti-tumor effects of RMAs, *e.g.* Bp, as shown before for calmodulin inhibitors (Wei *et al.*, 1983) and selective RMA-induced concentration-dependent MDR tumor-cell growth inhibition or stimulation. At this point the question remains to be answered whether the whole range of RMA-induced MDR-specific growth perturbations occur in human tumor cells with clinically relevant levels of MDR.

There is a discrepancy between our present results and those of Warr *et al.* (1986), who concluded that several CHO cell lines, but not $CH^R C_5$ cells, are hypersensitive for Vp. We have shown that Vp in $CH^R C_5$ cells offers the most extreme example of collateral sensitivity of MDR cells for RMAs. This discrepancy might be explained by differences in culture conditions (with or without prolonged exposure to colchicine) as indicated above.

In sum, no correlation was found between toxicity of RMAs and their ability to potentiate Dx sensitivity. Among the 4 compounds studied, the Vp analogue Ro was the most active in reversing Dx resistance in 2 MDR cell lines. Tp was found to affect Dx-sensitivity in a parental but not in the high-level MDR cell line. We also found that several MDR cells showed increased sensitivity to several, but not all, RMAs, and that this might be of use for clinical purposes. Lastly, some compounds—*e.g.*, cyclosporin A—show a concentration-dependent stimulation of MDR cell growth that should be taken into consideration in the planning of clinical trials with such drugs.

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