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ACTION OF CALCIUM ANTAGONISTS ON MULTIDRUG RESISTANT CELLS

SPECIFIC CYTOTOXICITY INDEPENDENT OF INCREASED CANCER DRUG ACCUMULATION

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Abstract—Previous studies have shown that calcium channel blockers can overcome, at least partially, multidrug resistance (MDR). This study was undertaken to attempt to determine the mechanisms whereby these agents bring about this effect. Their influence on the uptake and retention of several cancer drugs and on the toxic actions of these compounds was assessed employing MDR cell lines from several species. The wild-type drug sensitive parent cells proved to be more susceptible than the multidrug resistant variants to the effects of calcium channel blockers on cancer drug accumulation. This was shown for verapamil, nifedipine and the calmodulin inhibitor trifluoperazine acting on human, mouse and Chinese hamster ovary (CHO) cell lines. The enhancement of drug accumulation by calcium antagonists was similar to that caused by non-ionic detergents. Furthermore, verapamil was unable to alter $^{45}Ca^{2+}$ accumulation in sensitive or resistant cells, suggesting that these agents act in a calcium-independent manner. Verapamil accumulation in multidrug resistant cells was reduced compared to sensitive cells. In spite of this reduced accumulation, however, verapamil alone was much more toxic to multidrug resistant cells than to the sensitive cells. This suggests that calcium channel blockers are specifically toxic to MDR cells by virtue of an interaction with the MDR cell surface distinct from that involved in promoting cellular accumulation.

Multidrug resistance (MDR) is a complex phenotype of resistance to a variety of cancer drugs which may arise upon exposure of cells, either *in vivo* or *in vitro*, to these pharmacological agents [1]. MDR cells exhibit resistance to, and reduced cellular accumulation of, the selecting agent as well as cross-resistance to a number of structurally and functionally unrelated compounds [1, 2]. MDR cells may also display hypersensitivity (collateral sensitivity) to certain other drugs [3]. Although the mechanism by which drugs are excluded from MDR cells is not yet fully understood, a large plasma membrane glycoprotein (P glycoprotein), which is overexpressed [4] by virtue of amplification of its genes [5], is likely to be involved [6, 7].

The development of resistance to drugs employed in combination chemotherapy is a serious clinical problem, and means of overcoming it have been sought. It has been reported that certain calcium channel blockers such as verapamil and nifedipine [8–14] and the calmodulin antagonist trifluoperazine [15–17] are able to overcome or at least partially circumvent MDR in certain animal and human cells. Both verapamil [18] and nifedipine [19] have been shown to be specific inhibitors of the slow voltage-dependent calcium channel in mammalian cardiac muscle fibres. Trifluoperazine binds to calmodulin in a calcium-dependent manner and is, therefore, an inhibitor of calmodulin-dependent processes [20]. The ability of these agents to increase the cellular accumulation and cytotoxicity of vincristine by vincristine-resistant P388 leukemia has been attributed to an inhibition of efflux of that drug via a calcium-mediated membrane perturbation [11, 15, 17]. Kessel and Wilberding [21] proposed that verapamil is a substrate for the active efflux mechanism of P388 cells and thereby competitively inhibits daunomycin efflux. Conversely, Fojo *et al.* [8] reported verapamil-enhanced uptake of drug by human KB cells with no apparent effect on efflux. An alternative hypothesis is that calcium channel blockers act by modulating the inhibition of nucleic acid synthesis caused by cancer drugs [22] rather than by increasing their intracellular levels. Moreover, although Ganapathi *et al.* [16] observed a correlation between the potency of calmodulin inhibitors and their ability to restore adriamycin responsiveness in resistant P388 cells, Tsuruo *et al.* [17] did not find this correlation with calcium channel blockers.

It is clear that the mode of action of these calcium antagonists on drug resistance is not yet well understood. For example, it remains to be determined whether or not calcium channel blockers and calmodulin inhibitors act via the inhibition of calcium-related processes or in some non-specific calcium-independent manner. For the purpose of further clarification of the role that verapamil and other calcium channel blockers play in circumventing

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MDR, their effects on drug accumulation and cytotoxicity were investigated using different MDR cell types including Chinese hamster ovary (CHO) cells, murine L cells and human leukemia cells. The results indicate that wild-type cells are, in fact, more susceptible to the effects of calcium channel blockers with respect to enhanced cancer drug accumulation. However, the cytotoxic action of calcium channel blockers appears to be a direct action on the surface of MDR cells and not primarily due to an enhanced accumulation of other drugs. Preliminary results of this work have been presented elsewhere [23].

MATERIALS AND METHODS

Materials. [G-^{3}H]Vinblastine sulfate (19.0 Ci/mmol) was from Amersham, Arlington Heights, IL. The following were obtained from New England Nuclear, Boston, MA: [^{3}H(G)]daunomycin (4.08 Ci/mmol), [ring C, *methoxy*-^{3}H]colchicine (38.6 Ci/mmol), [*N-methyl*-^{3}H]verapamil (61.65 Ci/mmol) and $^{45}Ca^{2+}$ as $CaCl_2$ (35.73 mCi/mg). Verapamil hydrochloride, vinblastine sulfate, daunomycin hydrochloride, nifedipine, trifluoperazine, Triton X-100, Lubrol WX and 2,4-dinotrophenol were obtained from the Sigma Chemical Co., St. Louis, MO; diphenylamine and acetaldehyde were from Fisher Scientific, Don Mills, Ontario. Growth medium was obtained from the Ontario Cancer Institute Media Department, Toronto, Ontario; fetal calf serum was from Flow Labs Inc., Mississauga, Ontario.

Cell culture. The drug-sensitive wild-type CHO cells (Aux-B1) and drug-resistant mutants (B30) have been described [24]; B30 cells were grown in the presence of 30 μg/ml colchicine. Transformed murine L cells (LTA cells) and drug resistant mutants derived from them (LC1 cells) were those described previously [25]. The LC1 cells were grown in the presence of 1 μg/ml colchicine. Human CCRF-CEM cells and a corresponding vinblastine-resistant mutant, C/VLB^R, have been described [26]; C/VLB^R cells grow in 1 μg/ml of vinblastine. All cells were grown in complete alpha minimum essential medium with added nucleosides and ribonucleosides [27] plus 10% (v/v) fetal calf serum except for mouse cells which were grown in the absence of nucleosides and ribonucleosides. Cells were subcultured every 3–4 days to maintain exponential growth. Drug-resistant cells were transferred to drug-free medium 5–6 days before experiments were performed.

Drug accumulation and efflux. CHO and L cells were subcultured into 6 well Linbro dishes (Flow Labs Inc.) in 2 ml medium 2–3 days before experiments that were performed on cells in late-log stage of growth. Prior to experiments, growth medium was replaced with the same medium buffered with 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.2) instead of $NaHCO_3$. All experiments were carried out at 37° in a humidified air atmosphere. Effectors and tritiated drugs were added to a separate aliquot of HEPES-buffered medium containing 10% (v/v) fetal calf serum to the correct concentration from stock solutions (all were in distilled water with the following exceptions: nifedipine was in dimethyl sulfoxide, [^{3}H]colchicine and [^{3}H]verapamil were in ethanol, and [^{3}H]vinblastine was in methanol).

Experiments were initiated by aspirating the medium from the wells and adding 2.5 ml of medium at 37° containing the appropriate effector and drugs. Verapamil, daunomycin and vinblastine were added at 0.1 μCi/ml, and colchicine at 0.3 μCi/ml. The concentration of cold carrier drug is indicated in the text and figure legends. Plates were then incubated for the times indicated in the figures. To terminate the experiment, medium was aspirated, and the cell layer was rapidly rinsed with 3 × 5 ml phosphate-buffered saline (PBS) at room temperature. Figure 1 compares efflux of daunomycin from cells washed with PBS either at room temperature or at 4°. We felt that this was the experiment that could be most affected by temperature of washing. Because the results were similar at both temperatures, washing at room temperature was employed. Following the washing procedure, 1 ml of distilled water was added to each well. After cell lysis, the water and cell debris were transferred to scintillation vials. The well was rinsed with another 1-ml aliquot which was added to

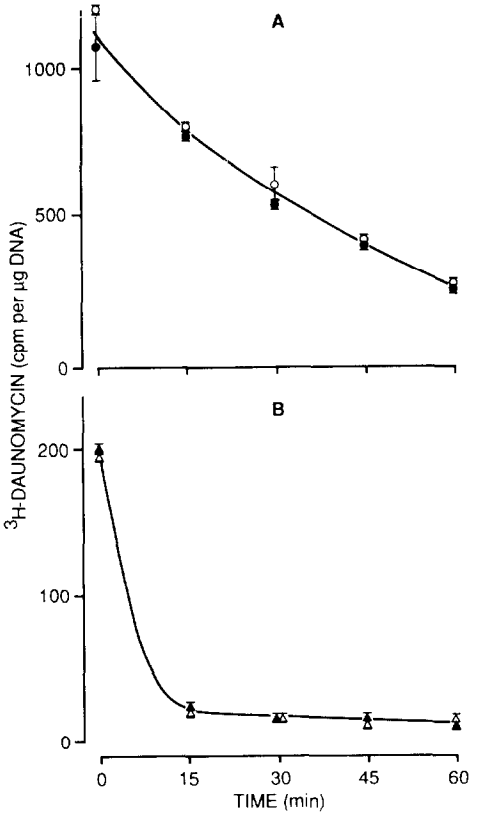

Fig. 1. Efflux of daunomycin from daunomycin-loaded sensitive (B1, A) (○, ●) and resistant (B30, B) (△, ▲) cells using different washing conditions: PBS at 4° (open symbols) or at room temperature (closed symbols). Cells were loaded with [^{3}H]daunomycin by incubating for 1 hr in the presence of 1 mM dinitrophenol. This and subsequent figures are representative of two or more experiments. Points are means of two measurements which are indicated by bars. Specific activities of tritiated drugs employed are indicated in Materials and Methods.

the scintillation vials. Ten millilitres of xylene-based scintillation mixture was added (PCS-II, Amersham), and the samples were counted in a Beckman model LS 8100 liquid scintillation counter. The ^3H counting efficiency was between 30 and 40%. In experiments with colchicine or vinblastine, all plates and bottles with medium were covered with aluminum foil to minimize photochemical decomposition of the drugs. Experiments with $^{45}Ca^{2+}$ were carried out in a similar manner except that $^{45}Ca^{2+}$ was added at a radiochemical concentration of 1 μCi/2.5 ml medium; the calcium concentration was 1.5 mM. In all experiments, blanks were obtained by incubating an empty well with tritiated drug or $^{45}Ca^{2+}$-containing medium and washing as described. This value was subtracted from those obtained with cells.

In order to load the cells with daunomycin, cells were incubated as above at 37° in medium containing 1 μM daunomycin plus 0.1 μCi/ml [^{3}H]daunomycin plus 1 mM dinitrophenol. After 1 hr, this medium was removed and replaced with glucose-containing medium plus or minus 19.8 μM verapamil. After the times indicated in Fig. 3, the cells were rinsed with PBS, and the cellular [^{3}H]daunomycin content was determined as described above.

CCRF-CEM cells grown in suspension were centrifuged at 500 *g* for 10 min at 25° and resuspended in HEPES-buffered medium at a concentration of approximately 2×10^7 cells/ml. A 100-μl aliquot of cells was added to 900 μl of medium with tritiated drug plus effector and incubated at 37° for the times indicated. Experiments were terminated by the addition of 2 ml of cold PBS followed by centrifugation (2 min). The pellet was washed with 2×2 ml PBS. Finally, the cell pellet was lysed and counted as described above.

Growth assays. The abilities of drug-sensitive and -resistant cells to grow in the presence of cytotoxic drugs were assessed by the determination of plating efficiencies. For continuous exposure experiments, cells were seeded at approximately 100, 200 and 400 per plate containing medium with the appropriate drug concentration. To determine short-term cytotoxicity, cells were exposed to medium with the appropriate drug concentrations for 1 hr. Following trypsinization, the cells were seeded as described above in drug-free growth medium. In both cases, cells were allowed to grow until colonies of 50 or more cells were observed in the control plates without drugs (approximately 7–8 days for B1 cells; 8–9 days for B30 cells). Medium was removed and colonies were fixed and stained in 0.5% methylene blue (Fisher Scientific) in 50% methanol and counted.

Determination of DNA content. To quantitate cells, DNA content was determined by the Leyva and Kelley [28] modification of the Burton [29] diphenylamine colorimetric assay. Briefly, cells were lysed in 2 ml H_2O and centrifuged at 8000 *g* for 20 min at 4°. The pellet was suspended in 200 μl of 10 mM Tris–HCl, pH 7.4, and 200 μl of 0.4 M perchloric acid followed by incubation at 4° for 30 min. Samples were centrifuged for 15 min, and the resultant pellet was digested in 250 μl of 1 M perchloric acid at 70° for 30 min. After cooling, 0.5 ml of the acidic diphenylamine-acetaldehyde reagent was added, and samples were incubated overnight (approximately 15 hr) at 37°. After centrifugation for 15 min, the A_{600} of the supernatant fraction was determined. Standard curves were constructed using calf thymus DNA Type I (Sigma) and 100 μg bovine serum albumin (Sigma) as carrier protein.

RESULTS

Uptake of ^{3}H-labeled drugs in absence of effectors. Figure 2 shows the uptake of daunomycin, vinblastine and colchicine by sensitive (B1) and resistant (B30) CHO cells. This clearly illustrates that the cells selected for resistance to one drug had impaired permeability to that drug, as well as to several others: although the resistant (B30) cells were selected for resistance to colchicine, their accumulation of both daunomycin and vinblastine was reduced greatly compared to the wild-type cells. Wild-type cells took up considerably less vinblastine (0.14 pmol at 30 min) than daunomycin (0.64 pmol at 30 min) or colchicine (0.52 pmol at 30 min). The accumulation of vinblastine and daunomycin by resistant (B30) cells was negligible; that of colchicine was slightly greater (0.15 pmol at 30 min).

Effect of verapamil on drug accumulation and retention. Figure 3A shows that the uptake of [^{3}H]daunomycin by CHO cells over time was enhanced by 19.8 μM verapamil. This effect, however, was not limited to the MDR cells. The observed stimulation of daunomycin uptake by sensitive cells indicates that the calcium channel blockers can act in a non-discriminatory manner with both cell types rather than specifically with MDR cells. The efflux of [^{3}H]daunomycin from daunomycin-loaded sensitive and resistant cells is shown in Fig. 3B. The presence of 19.8 μM verapamil in the

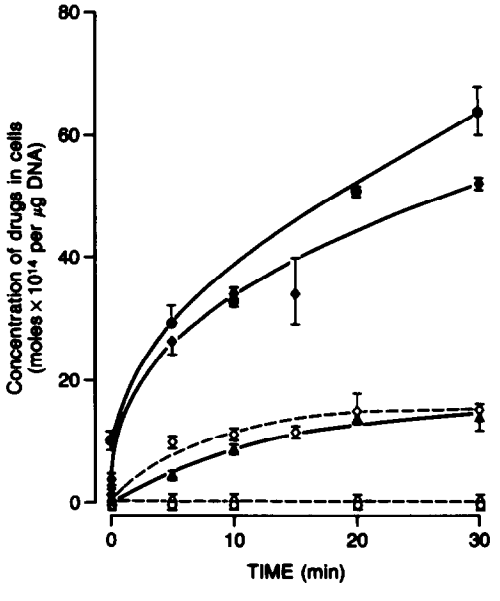

Fig. 2. Time course of uptake by CHO cells of: 1 μM daunomycin (B1, ●; B30, ○); 1 μM colchicine (B1, ◆; B30, ◇) and 50 nM vinblastine sulfate (B1, ▲; B30, △).

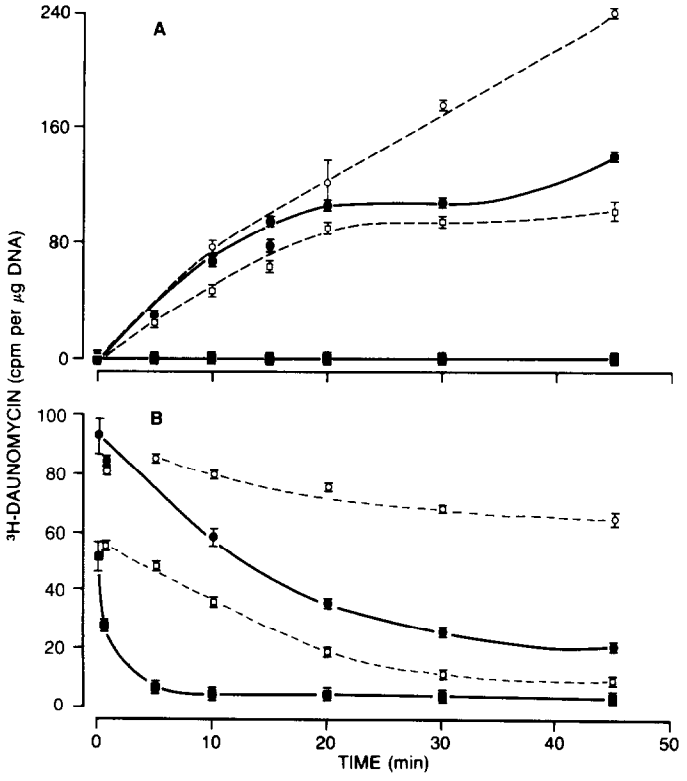

Fig. 3. Influence of verapamil on: (A) accumulation and (B) retention of 1 μM daunomycin by CHO cells (B1, ○, ●; B30, □, ■) in the presence (– – –) and absence (——) of 19.8 μM verapamil. To measure efflux of daunomycin, cells were loaded with [^{3}H]daunomycin in the presence of 1 mM dinitrophenol for 1 hr.

medium was clearly able to interfere with the efflux of drug in both cell lines. Verapamil reduced the apparent initial rate of efflux from 7.2 cpm/μg DNA/min to 1.5 and from 23 to 3 for sensitive (B1) and resistant (B30) cells respectively. This result is consistent with other studies which suggested that verapamil is able to inhibit efflux of daunomycin [17] as well as vincristine and adriamycin [11, 13] from multidrug resistant P388 leukemia cells. However, the present results indicate that verapamil augments the retention of daunomycin by both cell lines and, in fact, to a greater degree by the sensitive (B1) cells. Thus, verapamil acts to enhance uptake of daunomycin as well as interfere with its efflux from cells. The kinetics, in the case of sensitive cells, suggest that the reduced efflux, detectable within the first 5–10 min could account for the increased uptake which is first apparent at about 20 min. As for the resistant cells, major effects on both efflux and uptake were seen within the first 5 min. More rapid kinetic analyses are required to determine which effect may be primary.

Figure 4 shows the effect of increasing verapamil concentrations on the accumulation of daunomycin, vinblastine and colchicine by CHO cells. There was a marked augmentation of accumulation of all these drugs by sensitive (B1) cells at very low verapamil concentrations (2–5 μM for vinblastine and daunomycin; 10 μM for colchicine). Over this range there was very little influence on the uptake of these drugs by resistant (B30) cells: stimulation of daunomycin and vinblastine accumulation occurred only at much higher verapamil concentrations. In the case of colchicine, there was no increase in uptake by resistant cells with any verapamil concentration. The ability of verapamil to affect vinblastine and daunomycin accumulation but not colchicine by resistant (B30) cells suggests that there must be some differences in the modes of accumulation of these drugs by cells.

The marked sensitivity to verapamil of drug accumulation by sensitive as opposed to resistant cells was not limited to CHO cells. As shown in Fig. 5, similar although less pronounced effects were observed with human and mouse cells. Figure 5A shows that low concentrations of verapamil promoted daunomycin uptake by sensitive human leukemia (CCRF/CEM) cells to a greater extent than by a resistant variant (C/VLB^R). Similarly, parent murine L cells (LTA) showed a greater sensitivity to low verapamil doses than the corresponding mutant, LC1 (Fig. 5B). It is interesting to note that the maximum achievable plasma concentration of verapamil *in vivo* is approximately 2 μM [10]. In our experiments, at this low concentration only the sensitive cells of each type were affected by verapamil.

Two other agents, nifedipine, a calcium channel blocker, and trifluoperazine, a calmodulin antagonist, were tested for their effects on drug accumu-

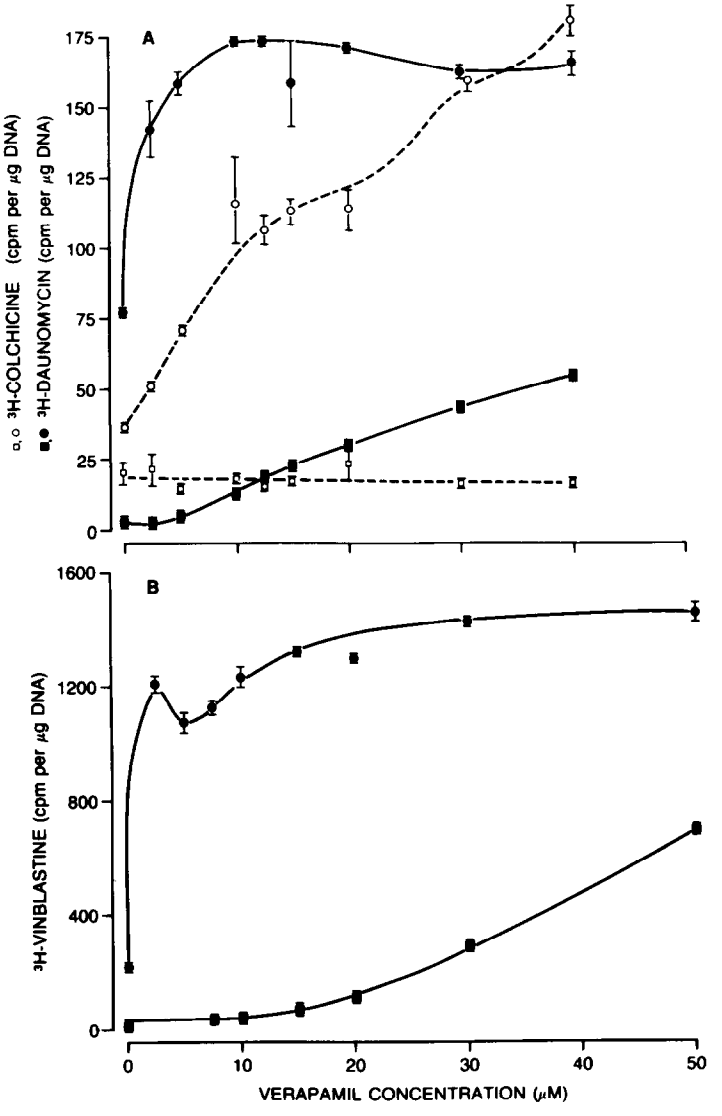

Fig. 4. Effect of increasing verapamil concentrations on the uptake (1 hr) by CHO cells of: (A) 1 μM daunomycin (B1, ●; B30, ■); 1 μM colchicine (B1, ○; B30, □) and (B) 50 nM vinblastine sulfate (B1, ●; B30, ■).

lation by CHO cells. Figure 6 illustrates these effects. As with verapamil, these agents enhanced [^{3}H]daunomycin accumulation by sensitive (B1) cells to a greater extent than by resistant (B30) cells. There was more than a 2-fold increase in daunomycin accumulation by sensitive (B1) cells at 100 μM nifedipine relative to cells incubated without nifedipine; there was essentially no effect on the resistant (B30) cells. The response of both cell lines to trifluoperazine was similar to their response to verapamil: low concentrations of trifluoperazine enhanced daunomycin accumulation by sensitive (B1) cells, whereas much higher concentrations were required to achieve increased daunomycin levels in resistant (B30) cells. Thus, the susceptibility of sensitive cells to the effects of calcium channel blockers was not limited to a single cell type or agent.

This apparent lack of specificity suggested the possibility that these agents may have a generalized effect on the cell membrane. To investigate this possibility, the influence of non-ionic detergents on daunomycin accumulation by CHO cells was tested. Both Triton X-100 and Lubrol WX markedly enhanced the accumulation of [^{3}H]daunomycin by sensitive (B1) cells at very low concentrations (Fig. 7). Resistant (B30) cells, on the other hand, were less susceptible to these detergents: the dose–response curves were sigmoidal with maximal effects observed only at 5-fold greater concentrations than with sensitive cells. The similarity of these dose–response curves to those with verapamil tends to confirm that the calcium channel blockers can act in a generalized, detergent-like manner.

$^{45}Ca^{2+}$ uptake. The above findings made it seem

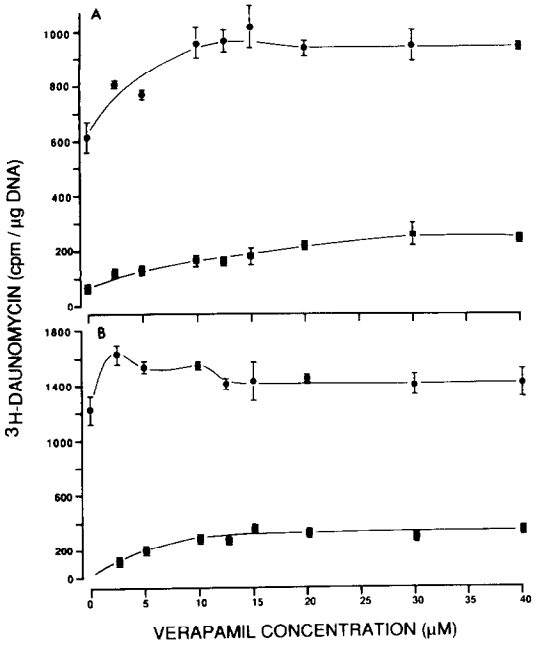

Fig. 5. Influence of increasing verapamil concentration on the uptake (1 hr) of 1 µM daunomycin by: (A) human leukemic lymphoblasts (CCRF/CEM, ●; C/VLB^R, ■) and (B) transformed murine L cells (LTA, ●; LC1, ■).

unlikely that calcium channel blockers were enhancing drug accumulation by virtue of their specific interactions with voltage-dependent calcium channels. To test this directly, sensitive and resistant cells were equilibrated with $^{45}Ca^{2+}$ plus or minus verapamil (data not shown). $^{45}Ca^{2+}$ entered both cell types at similar rates in the presence or absence of 10 µM verapamil. In fact, verapamil was ineffective over a wide range of concentrations. These data suggest that, indeed, calcium channel blockage is not the main mode of action of these calcium channel blockers in enhancing drug accumulation. This result may not be surprising since the voltage-sensitive calcium channels which the calcium channel blockers inhibit might not be expected to be constituitively expressed or active in cultured non-muscle cells.

Accumulation of [^{3}H]*verapamil by CHO cells.* The possibility that MDR may exclude calcium blockers as well as other agents to which they are cross-resistant was suggested by the fact that they were found to be less sensitive to the action of calcium channel blockers on drug accumulation. Therefore, the abilities of both sensitive and resistant cells to accumulate [^{3}H]verapamil were compared. Figure 8 shows that the accumulation of verapamil was like that of cancer drugs to which resistant (B30) cells are cross-resistant: that is, at 1 µM verapamil there was virtually no accumulation of verapamil by resistant (B30) cells, whereas sensitive (B1) cells accumulated this drug to a much greater extent. This also occurred at much higher verapamil concentrations: at 230 µM verapamil, resistant (B30) cells accumulated about 50% as much verapamil as sensitive (B1) cells (Fig. 8 inset).

Effects of calcium channel blockers on plating efficiencies. To further examine the possibility of cross-resistance to verapamil, continuous and short-term plating experiments in the presence of verapamil alone were carried out. The results emphatically showed that the MDR cells were not cross-resistant to verapamil. Figure 9A indicates that concentrations of up to 500 µM verapamil had little effect on the growth of sensitive (B1) cells, whereas resistant (B30) cells were unable to grow after 1-hr exposure to 300 µM verapamil (Fig. 9B). Similarly, continuous exposure experiments showed that 2 µM verapamil was toxic to MDR cells, whereas sensitive (B1) cells can grow in up to 80 µM verapamil (data not shown). Thus, verapamil is an agent to

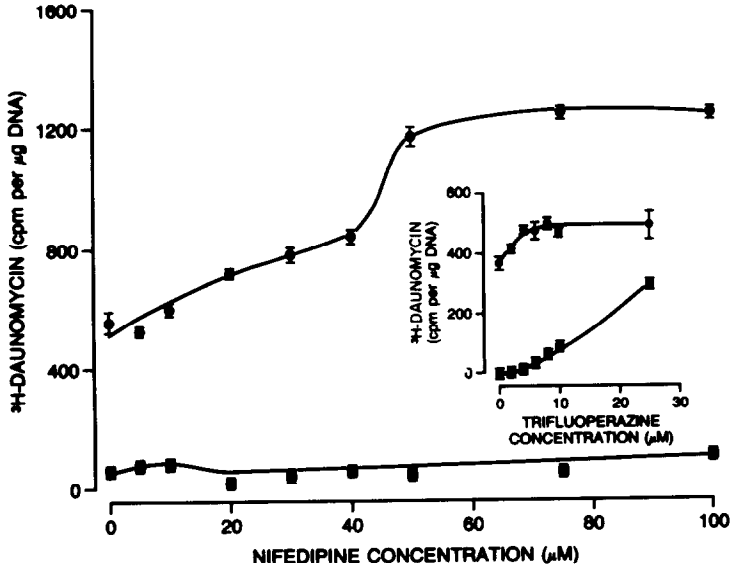

Fig. 6. Uptake of 1 µM daunomycin by CHO cells (B1, ●; B30, ■) in the presence of increasing concentrations of nifedipine and trifluoperazine (inset).

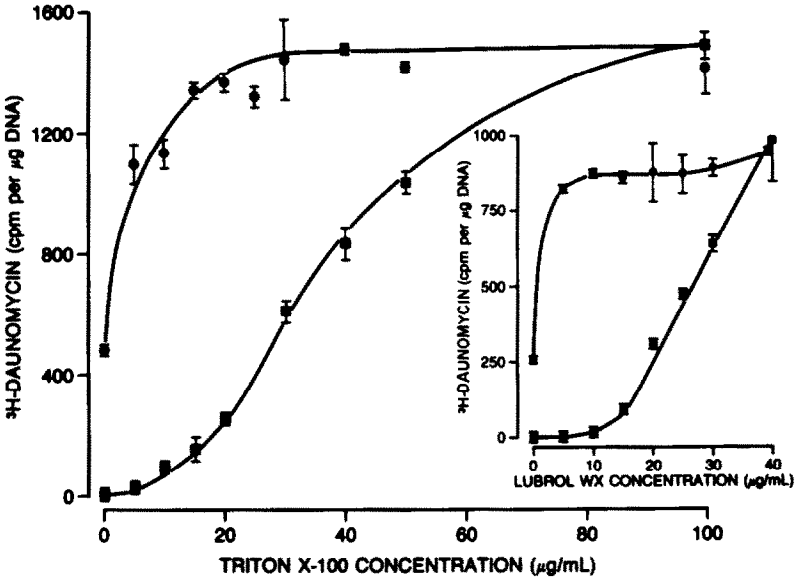

Fig. 7. Effect of the non-ionic detergents Triton X-100 and Lubrol WX (inset) on accumulation (1 hr) of 1 μM daunomycin by CHO cells (B1, ●; B30, ■).

which these MDR cells are collaterally sensitive rather than cross-resistant. This acute sensitivity to verapamil occurred in spite of reduced accumulation of high or low concentrations of verapamil by these resistant (B30) cells (Fig. 8).

To determine the effect of verapamil plus daunomycin on cell growth, plating efficiencies in the continuous presence of both drugs were determined. Figure 10 shows growth curves of sensitive (B1) cells and resistant (B30) cells in increasing concentrations of daunomycin with addition of 0, 0.1 or 1.0 μM verapamil. A 0.1 μM concentration of verapamil shifted the curves relating cell growth to daunomycin concentration of both sensitive (B1) and resistant (B30) cells slightly to the left: 0.1 μM verapamil reduced the IC_{50} (concentration of drug which is inhibitory to growth of 50% of cells) from 0.038 to 0.031 and from 3.8 to 3.2 μM daunomycin for sensitive (B1) and resistant (B30) cells respectively. Verapamil at 1.0 μM shifted the growth curve of resistant (B30) cells in daunomycin such that cell kill occurred at a much lower concentration than with daunomycin alone. In contrast, this shift was much smaller with sensitive (B1) cells: a 12-fold reduction in IC_{50} of daunomycin (from 3.2 to 0.3 μM) in resistant (B30) cells was observed in the presence of 1 μM verapamil as compared with a 2.4-fold reduction (from 0.031 to 0.013 μM) for sensitive (B1) cells. These results confirm that verapamil, in spite of reduced accumulation by the MDR cells, is more toxic in combination with daunomycin to resistant (B30) cells than to sensitive (B1) cells. In light of the

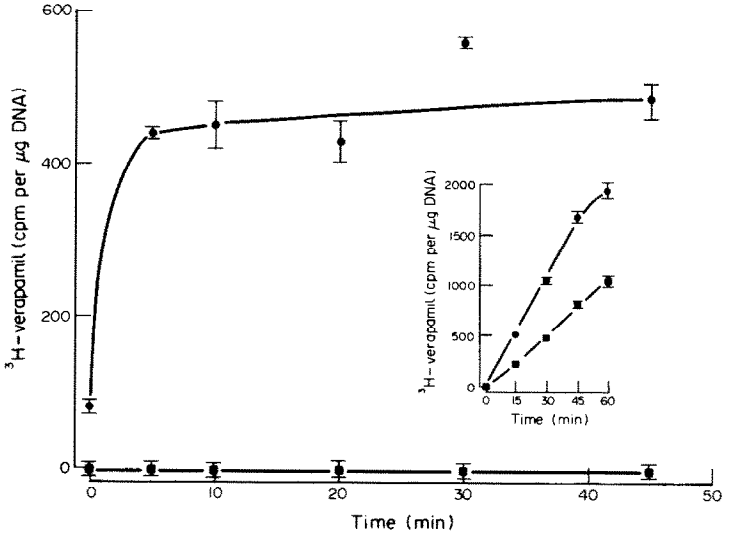

Fig. 8. Time course of accumulation of 1 and 230 μM verapamil (inset) by CHO cells (B1, ●; B30, ■).

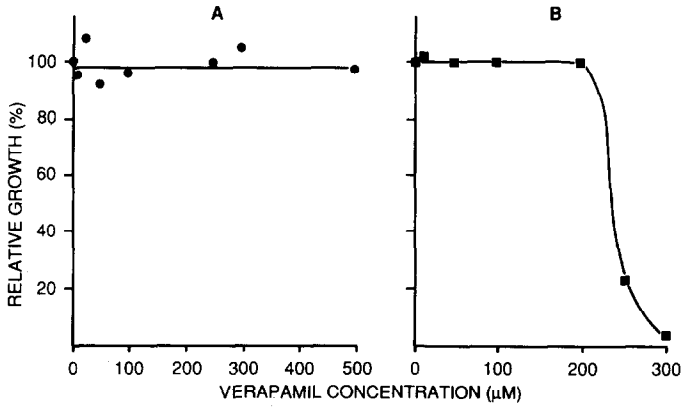

Fig. 9. Influence of increasing concentrations of verapamil on the plating efficiencies of: (A) sensitive (B1, ●) and (B) resistant (B30, ■) cells after 1-hr exposure. Absolute plating efficiencies are: sensitive (B1) cells, 58% and resistant (B30) cells, 60%.

growth assays in the presence of verapamil alone it seems likely that verapamil itself is primarily responsible for cytotoxicity to resistant (B30) cells, whereas verapamil probably does augment the toxic effect of daunomycin in sensitive cells by increasing its accumulation. Verapamil and nifedipine, therefore, appear to exert a specific cytotoxic action on MDR cells which is of greater magnitude than that of their effect on the uptake of other drugs by these cells.

DISCUSSION

Several characteristics of MDR were revealed in this study. The results confirm other findings which reported that calcium channel blockers enhance accumulation of cancer drugs in MDR cells and, in combination with them, are more toxic than the cancer drugs alone [11, 13, 17]. However, this study shows that augmented accumulation of cancer drugs was most prominent in wild-type rather than MDR murine, human and CHO cells. Therefore, promotion of drug accumulation by calcium channel blockers was not a specific effect on the MDR mechanism and, in fact, was similar to the non-specific membrane perturbing effects of non-ionic detergents. In addition, the lack of influence of calcium channel blockers on $^{45}Ca^{2+}$ equilibrations suggested that these agents are acting in a calcium-independent manner. Although it can be argued that Triton X-100 is an inhibitor of calmodulin-dependent phosphodiesterase [30], Lubrol WX requires a much higher concentration to cause 50% inhibition of this calmodulin-dependent phosphodiesterase (222 μg for Lubrol WX as compared with 9 μg for Triton X-100) [30] and yet has the same overall effect on drug accumulation as Triton X-100. These observations taken together suggest that, in this instance, the calcium channel blockers under study were acting in a manner different from their ability to inhibit voltage-sensitive calcium channels. This was essentially confirmed by experiments in which $^{45}Ca^{2+}$ equilibration by sensitive and resistant cells was measured: verapamil was unable to alter $^{45}Ca^{2+}$ accumulation in either cell line. This is not a very surprising result since Fine *et al.* [31] have reported the absence of nitrendipine-sensitive calcium channels in adriamycin-resistant CHO cells.

The relative degree of accumulation of verapamil itself by sensitive and resistant cells was similar to that of cancer drugs. However, in spite of its impaired

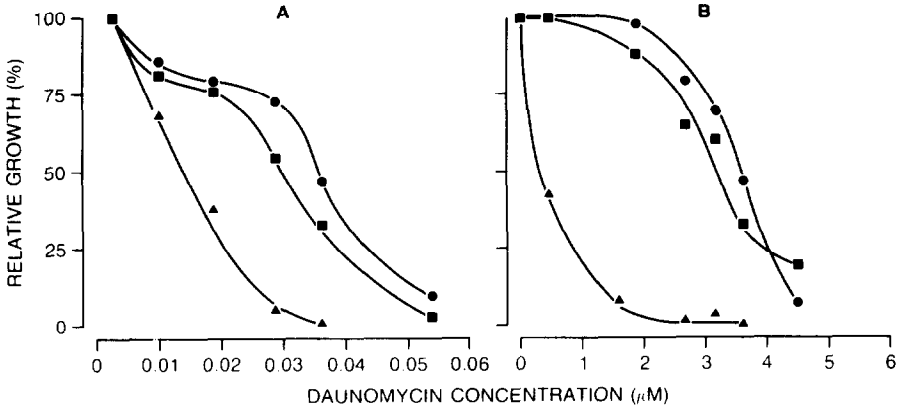

Fig. 10. Plating efficiencies of: (A) sensitive (B1) and (B) resistant (B30) CHO cells in daunomycin plus 0 (●), 0.1 μM (■) or 1.0 μM (▲) verapamil. Absolute plating efficiencies are: sensitive (B1) cells, 61% and resistant (B30) cells, 70%.

accumulation by resistant cells, verapamil is very toxic to them. The observation that MDR cells were collaterally sensitive to verapamil even though its cell-association was diminished strongly suggests that calcium channel blockers may exert their cytotoxic action on MDR cells by virtue of a specific interaction with their surface and not primarily by elevating the amounts of other drugs in the cell.

The collateral sensitivity of MDR cells to verapamil has not been observed in all MDR cell lines. For example, Beck [32] reported only a slight inhibition (10%) of growth of vinblastine resistant CCRF/CEM cells at 10 μM verapamil. The cytotoxicity of calcium antagonists to B30 cells may be a reflection of their very high level of pleiotropic resistance (approximately 1000-fold to colchicine). We intend to test this postulate using a series of increasingly colchicine-resistant CHO cells. However, Twentyman *et al.* [33, 34] have reported recently that a multidrug resistant small cell lung cancer line also exhibits acute sensitivity to verapamil. Hence, for at least some tumour cell types, these calcium channel blockers alone may have chemotherapeutic potential.

Another important consequence of the present study is the observation that colchicine, daunomycin and vinblastine respond differently to the effects of verapamil. Verapamil was able to enhance daunomycin, colchicine and vinblastine accumulation by sensitive (B1) cells. Daunomycin and vinblastine accumulation by resistant (B30) cells was also increased. However, this augmented accumulation occurred only at much higher verapamil concentrations than those required to stimulate accumulation of these drugs in sensitive cells. Furthermore, verapamil was completely ineffective in altering the accumulation of colchicine by resistant (B30) cells. Hence, these drugs probably exhibit some differences in their modes of accumulation by the cells. In fact, it is not difficult to envisage the necessity of separate routes of entry for these structurally dissimilar compounds. The ability of verapamil to enhance accumulation of vincristine to a much greater degree than that of adriamycin by cells of mice bearing resistant tumours has been reported by Tsuruo *et al.* [17, 35]. The same has been reported for both vinblastine and doxorubicin resistant CCRF/CEM cells [36].

In summary, this study has been able to dissect the effects of calcium channel blockers on the cellular cancer drug accumulation from their specific cytotoxic effects on MDR cells. Despite the fact that the mechanism responsible for the exclusion of cancer drugs from MDR cells also recognizes calcium antagonists, these agents are especially toxic. This implies a specific interaction between them and some constituent of the MDR cell membrane. Photoaffinity labeling experiments with certain calcium channel blockers have indicated that this is, in fact, the case.*

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