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Saturation reversal of the multidrug pump using many reversers in low-dose combinations

Elena Lyubimov, Lu-Bin Lan, Irina Pashinsky, Suhail Ayesh and Wilfred Donald Stein
Biochemistry Department, Silberman Institute of Life Sciences, Hebrew University, Jerusalem 91904, Israel.
Tel: (+972) 2-6585409; Fax: (+972) 2-6585440.

Multidrug resistance in cancer cells, in cell culture and in the clinic, is often associated with a membrane protein (the multidrug resistance pump or P-glycoprotein) that pumps out anti-cancer drugs as fast as they enter the cell. This pump is blocked by a range of well-known pharmaceuticals that reverse drug resistance. We have investigated whether effective reversal of drug resistance could be achieved by using many reversers together, each at a low dose relative to its maximal tolerated plasma level. We measured in cell culture, using resistant P388 cells in suspension, the extent of reversal of the accumulation of two labeled cytotoxins (vinblastine and daunomycin). We fitted the data to a modified Michaelis–Menten equation and extracted the half-inhibition constants for 18 reversers acting on the pump. We measured also the reversal of resistance in a cell growth assay using incorporation of labeled thymidine. We showed that these drugs in groups of up to 18 together, each drug being at a low dose, in many cases well-tolerated in humans, had additive effects so that the combination was as effective as any of the drugs present singly. This was the case both for reversal of cell accumulation and for the effects of cytotoxins on cell growth. Our data show that a low-dose multidrug approach to saturation reversal of the multidrug pump is feasible in cell culture and provide the initial experimental basis for the development of an effective regime of such combination reversal therapy.

Key words: Chemotherapy, drug accumulation, multidrug resistance, reversal.

Introduction

Resistance to the cytotoxic action of cancer chemotherapeutic drugs is often associated with the manifestation of the multidrug resistance (MDR) pump, both in cells in culture and in patients in the clinic.[1–6] The MDR pump is a well-characterized membrane-bound protein, with demonstrable action as an ATPase.[7–10] Its activity results in a marked reduction in the intracellular concentration of a wide range of compounds, these being the substrates of the pump. Overcoming the action of this pump in a clinically acceptable fashion would be an important addition to our weapons against cancer. The action of the MDR pump in reducing intracellular drug concentrations can be reversed by a large range of compounds, many of them being well-studied pharmaceuticals in clinical use for conditions other than cancer.[11] Some of these, such as verapamil,[12] quinidine[13] and cyclosporin A,[14] have already been used in phase I and II clinical trials, in attempts to reverse drug resistance, but the high plasma levels of reversers that are needed have limited the usefulness of this approach. Structure–activity analysis of MDR pump reversers has led to the development of new drug candidates, with improved capacity to reverse MDR.[15] We have been developing another approach, in which combinations of many known reversers can be used in an attempt to use the low specificity of the MDR pump to block it [Israel patent 108757 (24 February 1994) to WD Stein]. In this paper we show that reversers of the MDR pump have additive effects on one another, so that combinations of these reversers in sets of five, nine, 10 and 18, with each drug present at 1/5, 1/9, 1/10 or 1/18, respectively, of its effective concentration when present alone are able to bring about reversal of the pump. This result demonstrates that a low-dose multidrug approach to saturation reversal of the multidrug pump is, in principle, feasible (although whether it will succeed in practice will depend on rigorous testing, first in whole animals and then in clinical situations).

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Correspondence to WD Stein

Materials and methods

Chemicals and radiochemicals

Vinblastine, amiodarone, amitriptyline, chlorpromazine, diltiazem, dipyridamole, fluphenazine, pro-

gesterone, propafenone, β-propranolol, quinidine, reserpine, spironolactone, terfenadine, trifluoperazine, and triflupromazine, verapamil were from Sigma (Sigma, MO). Mefloquine, promethazine and tamoxifen were kind gifts from Dr H Ginsburg. Tritium-labeled compounds were: [G-^{3}H]vinblastine (21 Ci/mmol) and [G-^{3}H]thymidine (27 Ci/mmol) from Amersham Life (Amersham, UK), [G-^{3}H] daunomycin (3.9 Ci/mmol) was from NEN-DuPont (Boston, MA).

Cell culture and treatments

The P388 lymphoma cells used were a MDR strain, initially selected for resistance to adriamycin, a kind gift from Professor Avner Ramu of the Hadassah-Hebrew University Hospital and have been fully characterized.[16–18] They were grown in RPMI 1640 medium (Biological Industries, Kibbutz Beit Ha'emek, Israel) to which were added penicillin (100 units/ml), streptomycin (100 μg/ml), amphotericin (0.25 μg/ml), 10 μM β-mercaptoethanol and 0.2% (w/v) glucose, together with 10% fetal calf serum (Biological Industries), the whole being hereafter termed complete RPMI medium, in 5% CO_2/humidified air at 37°C, in suspension culture, in 260 ml plastic bottles (Nunc, Denmark). Before an experiment, cells were counted in an Analys Instrument (Sweden), model 134 cell counter and the cells resuspended in fresh medium at 1.5×10^6 cells per 0.4 ml medium for the subsequent transport assay. The cells were then held for at least 1 h at 37°C to regenerate their full MDR pump activity.

Measurement of drug uptake

An aliquot of 0.4 ml of a suspension of cells in complete RPMI was transferred to a siliconized microfuge tube and 40 μl of the working solution of ^{3}H-labeled cytotoxin (with sufficient cytotoxin to give a final concentration of vinblastine at 0.33 nM or daunomycin at 2 nM) containing the required concentration of reverser was added at zero time. Preliminary experiments showed that the most reproducible results were obtained at the plateaus of the cytotoxin accumulation curves which were reached by 40 min incubation at 25°C for vinblastine, but required 60 min at 37°C for daunomycin. Uptake of the cytotoxin was stopped by rapid centrifugation in the Beckmann model 11 microfuge at maximum speed for 1 min. The supernatant layer was immediately quantitatively removed by aspiration and the cell pellet resuspended in 0.5 ml 10% Triton X-100. The cell suspension was then transferred to vials for radioactivity counting as above. In all cases, aliquots of cells were incubated for comparable times with no reverser present and zero time uptakes performed by adding appropriate aliquots of pre-cooled labeled vinblastine solutions to pre-cooled cell suspension. The latter determination served to measure the amount of label trapped between the cells during centrifugation. It was generally some 5–10% of the maximum amount of label that was taken up during incubation in the presence of reverser. In all cases, aliquots of the loading solutions were taken for scintillation counting to enable the conversion of the data from d.p.m. per sample to fmol of cytotoxin accumulated per 10^5 cells.

Cell growth assays

Resistant P388 cells were grown as described above and regenerated by suspension in fresh complete RPMI before transferring to 24-well plastic dishes (Nunc) in equal aliquots of 0.9 ml containing 1.5×10^6 cells. To each well was added the desired concentration of vinblastine with or without the desired concentration of reverser. Dishes were incubated for 22–24 h at 37°C and then, with the cells still in the exponential growth phase, 0.5 μCi of radio-labeled thymidine added, the cells being further incubated for 1 h at 37°C. They were then washed once, immediately transferred to ice and four aliquots taken from each well into a 96-well plate for radioactivity counting using the Packard Matrix 96 Direct Beta counter. Data are obtained as c.p.m. per one-fourth well.

Results

We performed a large series of measurements of the values of the effectiveness of many reversers, all of them being well-known pharmaceuticals, widely-used in various clinical situations, using the well-characterised line of MDR P388 lymphoma cells.[16–18] For each reverser we determined the extent of vinblastine accumulation (at 0.33 nM in the external medium, during 40 min at 25°C) or of daunomycin accumulation (at 2 nM in the external medium, during 60 min at 37°C) as a function of reverser concentration, using a conventional drug accumulation assay. We fitted the data to an easily-derived equation that describes the effect of the inhibition of the MDR pump's activity (the equation being a modified form of the classical Michaelis–Menten equation), written as $U = U_o + U_m \times (R/K_i + R)$, where U

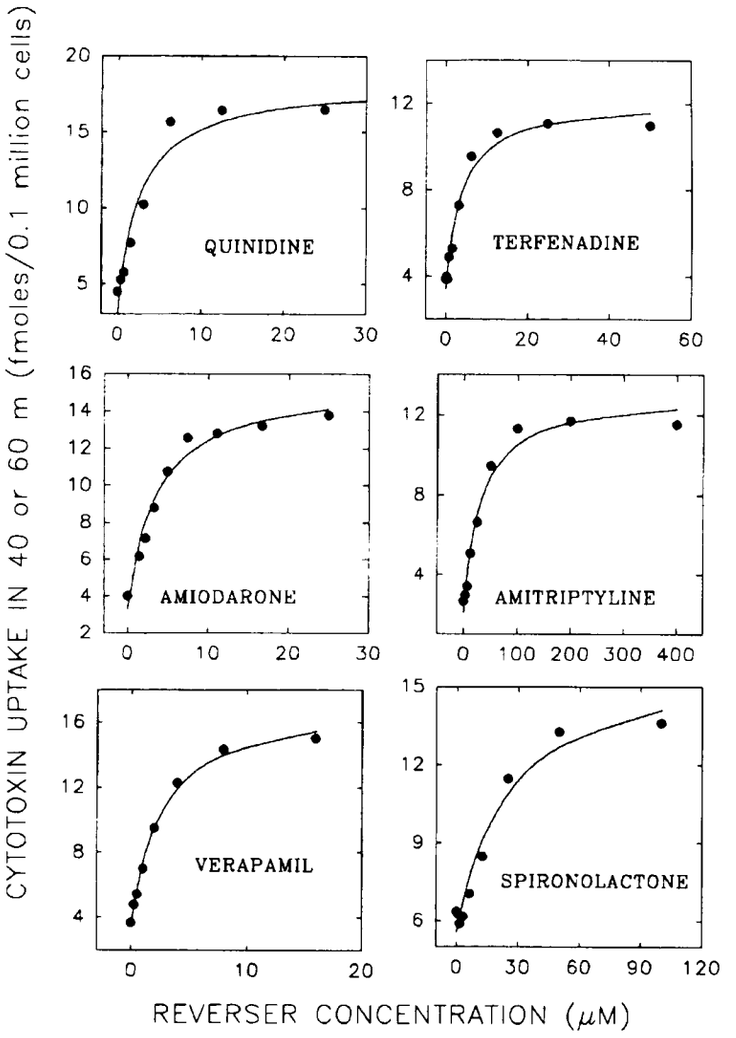

Figure 1. Effect of the reversers quinidine, terfenadine, amiodarone and amitriptyline on vinblastine accumulation (at 0.33 nM at 25°C) by MDR P388 lymphoma cells, and of verapamil and spironolactone on daunomycin accumulation (at 2 nM at 37°C) by these cells. Experiments were performed as in Methods. The lines are best-fits to the equation describing pump inhibition (see text). For the six curves shown, the values of K_i (that concentration of reverser that gives one-half the maximal degree of reversal) are 2.5 ± 1.0, 3.7 ± 0.8, 4.0 ± 1.4 and 30 ± 8 for quinidine, terfenadine, amiodarone and amitriptyline on vinblastine accumulation, and 2.6 ± 0.4 and 29 ± 9 for verapamil and spironolactone on daunomycin accumulation, respectively.

is the uptake of cytotoxin at concentration R of the reverser in the medium, U_o is the uptake in the absence, U_m is the increment in uptake in the presence of maximal concentrations, of the reverser, and K_i is the reverser concentration that gives one-half of the maximal increment in cytotoxin accumulation. (To check this equation, put R equal to zero when $U = U_o$, or let R become very large, when $U = U_m$, or put $R = K_i$, when the increment in accumulation will be half-maximal). Plotted in this form, each reverser gives a more-or-less comparable reversal profile. Figure 1 depicts six representative accumulation profiles of the many that we have determined. In a separate study (Lan *et al.*, submitted for publication) we have shown that the kinetic parameters that we obtain for such reversers are not different whether we measure their effects in our assays on vinblastine or on daunomycin accumulation). Table 1 records the calculated values of K_i, the concentration of reverser that gives one-half the maximal degree of reversal, for 18 reversers of various classes.

The data are reported in terms of the mean value of $K_i \pm$ SE for the number of determinations recorded in Table 1. Clearly, the various reversers vary widely in their effectiveness, K_i values ranging from

Table 1. Kinetic parameters for reversers acting on the MDR pump of drug-resistant P388 cells

Reverser (and pharmacological effect)[11]	K_i ± SE (μM)[b]	Maximal clinical plasma level (μM) and reference
Amiodarone (anti-arrhythmic)	3.26 ± 0.52 (4)	3.6[31]
Amitriptyline (anti-depressant)	42.8 ± 7.3 (3)	2.2[32]
Chlorpromazine (sedative)	17.0 ± 3.7 (4)	0.32[33]
Diltiazem (vasodilator)	19.1 ± 4.4 (4)	1.4[34]
Dipyridamole[a] (anti-coagulant)	2.27 ± 0.22 (3)	4.6[33]
Fluphenazine (tranquilizer)	20.0 ± 5.6 (9)	0.0064[36]
Mefloquine (anti-malarial)	2.07 ± 0.57 (5)	5.4[37]
Progesterone (hormone)	21.2 ± 10.7 (4)	4.1[25]
Promethazine (anti-histamine)	12.2 ± 2.6 (10)	0.22[38]
β-Propranolol (adrenergic blocker)	15.2 ± 3.3 (3)	0.50[39]
Quinidine (anti-arrhythmic)	5.47 ± 0.66 (4)	5.46[13]
Reserpine (anti-hypertensive)	0.62 ± 0.44 (3)	0.005[40]
Spironolactone[a] (diuretic)	18.4 ± 4.9 (4)	1.3[41]
Tamoxifen (anti-estrogen)	4.33 ± 0.60 (4)	3.7[26]
Terfenadine (anti-histaminic)	2.37 ± 0.45 (3)	2.6[42]
Trifluoperazine[a] (tranquilizer)	11.8 ± 0.5 (3)	0.27[27]
Triflupromazine (tranquilizer)	20.6 ± 3.8 (5)	—
Verapamil (anti-arrhythmic)	2.4 ± 0.5 (4)	3.8[12]

K_i, the reverser concentrations needed to block 50% of pump rate. Vinblastine uptakes at 25°C or daunomycin at 37°C[a]. [b]Numbers in parentheses indicate observations.

some 0.5 μM for reserpine through close to 50 μM for amitriptyline. The values of K_i that we report are in terms of the concentration of reverser *in the medium* that gives half-maximal reversal of the pump's action. They are thus merely operational descriptors of the effectiveness of the various modulators and do not bear on their site of action, i.e. within the cell or outside of it.[19] The maximal increment in accumulation, U_m, differs little between reversers but shows some day-to-day variance, perhaps dependent on the state of the cells.

Using this data base, we chose reverser concentrations that, for each drug in isolation, would give an approximately 2.5-fold increase (i.e. large but not maximal) in the amount of accumulation of our chosen chemotherapeutic drug, vinblastine. Figure 2 shows the data on the reversal of vinblastine accumulation in the resistant P388 cells for 10 of these reversers, taken separately and together, in two groups of five and in the full group of 10. The data are depicted as the means of three to 12 determinations in the various cases, with the calculated standard errors. The left-most column (labeled 'NO') depicts the uptake of vinblastine in the absence of reverser; columns 2–6, the uptake in the presence of five reversers (see legend for the meaning of the labels TE, MF, etc) in concentrations listed in the legend. The hatched column 7 (labeled Σ5) is the uptake in the presence of these five drugs taken together, but with each present at a concentration 1/5 of that used for columns 2–6. Columns 9–13 depict the uptake in the presence of five more drugs as listed, while the hatched column 14 (again labeled Σ5) shows the uptake for these five drugs, present together, each at a concentration 1/5 of that for columns 9–13. Finally, the solid column 8 (labeled Σ10) is the uptake in the presence of all 10 drugs, each now present at a concentration which is 1/10 of that used separately in columns 2–6 and 9–13. Clearly, these drugs are effective in additive fashion, taken five at a time or 10 at a time.

To test the applicability of this finding to another cytotoxic drug, we took 18 of the reversers in Table 1 and studied their action, separtely and together (in groups of nine and 18), in reversing the MDR pump's action on the cytotoxin daunomycin (at 2 nM at 37°C at 60 min). Figure 3 depicts the result of this experiment. The experiments in Figure 3(A and B) were done in parallel by two investigators, that in Figure 3(C) on the following day, by one of these investigators. Again, these 18 drugs taken together in nines and 18s act additively in blocking the MDR pump's action against daunomycin. Their effect, when present together, each at a concentration 1/9 or 1/18 of that used in the separate tests of their action (Figure 3, lowest panel), was as great as when each was present separately at the full concentration (Figure 3, upper two panels).

We considered it necessary to extend these findings also to the study of the effect of the reversers on the cytotoxins action on cell growth, rather than merely on the uptake of the cytotoxins. We therefore studied the effect of a range of these reversers, separately and together (in groups of five and 10), on the reduction of growth of resistant P388 lym-

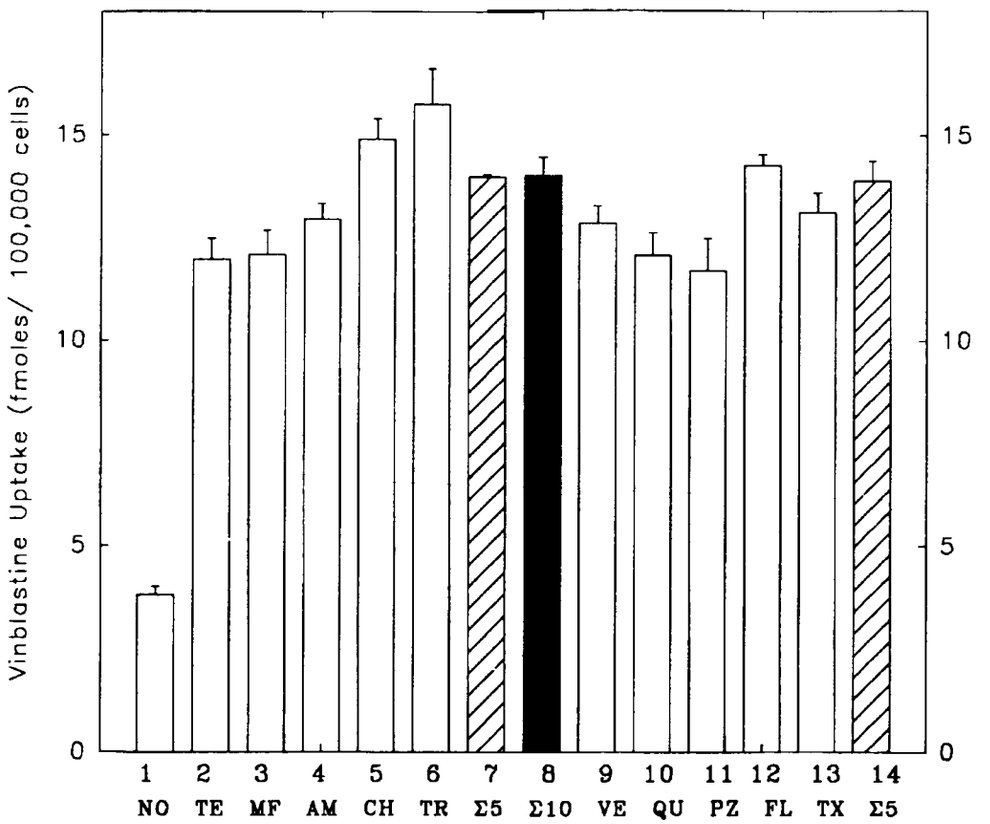

Figure 2. Effect of 10 reversers, separately and together, on the uptake of vinblastine by MDR P388 cells. Uptakes were measured as in Methods in the absence (column labeled NO) or in the presence separately of the reversers terfenadine (TE, 8.2 μM), mefloquine (MF, 6.4 μM), amiodarone (AM, 8.2 μM), chlorpromazine (CH, 22.7 μM), trifluprazine (TR, 16.4 μM), verapamil (VE, 10 μM), quinidine (QU, 50 μM), promethazine (PR, 61.7 μM), fluphenazine (FL, 12.5 μM) and tamoxifen (TX, 25.5 μM), or with five reversers present together (hatched columns, each referring to the five reversers to the left of that column) at 1/5 or with all 10 reversers present together (solid column) at 1/10 of the above concentrations of reversers.

phoma cells, as brought about by vinblastine. Figure 4 shows the effect of 10 drugs, separately and together, on increasing the measured cell growth in the presence of 30 nM vinblastine. (Figure 4 is representative of six such studies.) For each drug, we show cell growth (measured as the accumulation of labeled thymidine – see Methods) at the drug concentration listed in the legend, in the absence (total height of open bars) and in the presence of vinblastine (hatched area). Column 1 depicts residual cell growth measured in parallel in the absence of reversers, without (open area) and with 30 nM vinblastine (hatched area). We show also measured cell growth in the presence of a mixture of these 10 reversers (column 12), each reverser being present at 1/10 of the concentration that was used separately, cell survival being determined again in the absence (open area) and presence (hatched area) of 30 nM vinblastine. Once again, the effects of these 10 reversers are strictly additive. Taken together, each at 1/10 of an effective dose, they combine to bring about effective blocking of the MDR pump and hence an enhanced reduction in the ability of the cells to grow.

Discussion

Our data appear to be fully consistent with an approximately additive effect of these MDR pump-reversing drugs with each other, an effect seen in the case of the reversers taken in groups of five, nine, 10 or 18 together, using two cytotoxins and two modes of demonstrating drug resistance. The data do not suggest any clear super-additive effect of the drug mixtures, as might have been predicted from some

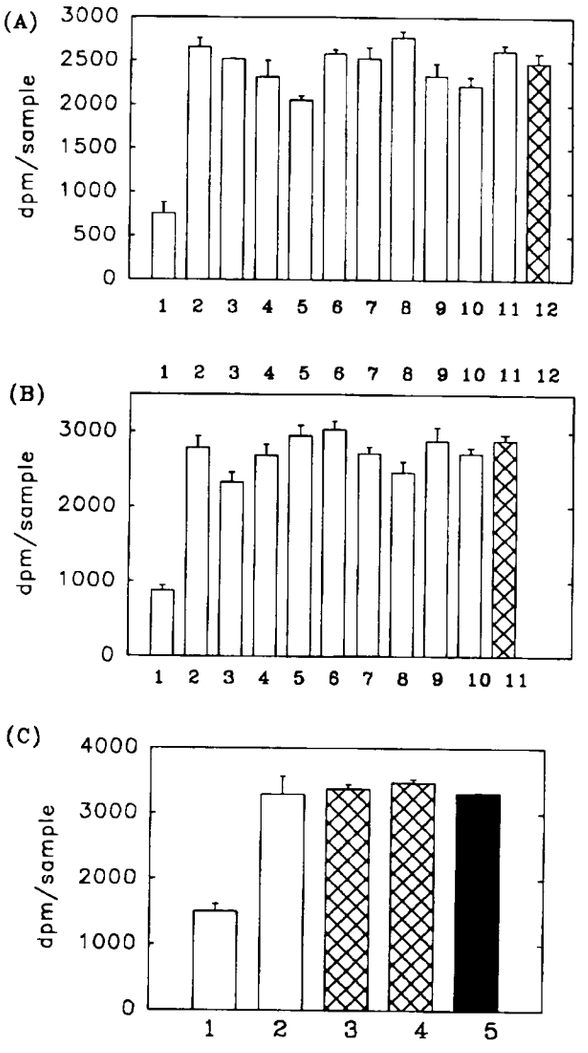

Figure 3. Effect of 18 drugs, separately and together, on the uptake of daunomycin by MDR P388 lymphoma cells. Uptakes were measured as in Figure 1 but incubation was at 37°C for 60 min and the microfuge tubes were not siliconized. Daunomycin at 2 nM. (A) Nine drugs as follows (column numbers followed by reverser and its concentration): 1, no reverser; 2, spironolactone 50 μM; 3, reserpine 1 μM; 4, diltiazem 20 μM; 5, progesterone 100 μM; 6, terfenadine 9 μM; 7, mefloquine 7 μM; 8, amiodarone 9 μM; 9, chlorpromazine 40 μM; 10, triflupromazine 40 μM; 11, tamoxifen 30 μM; 12, nine (i.e. all except tamoxifen) together at 1/9 the aforementioned concentrations. (B) Nine additional drugs as follows (column numbers followed by reverser and its concentration): 1, no reverser; 2, verapamil 10 μM; 3, quinidine 30 μM; 4, promethazine 62 μM; 5, fluphenazine 12.5 μM; 6, tamoxifen 25.5 μM; 7, amitriptyline 147 μM; 8, β-propranolol 75 μM; 9, dipyridamole 27 μM; 10, trifluoperazine 6 μM; 11, nine together at 1/9 the aforementioned concentrations. (C) Drugs in sets of nine and 18: column 1, no reversers; 2, verapamil at 10 μM; 3, drugs of (A) nine together at 1/9 concentration; 4, nine drugs of (B) together at 1/9 concentration; 5, all 18 drugs of (A) and (B) together at 1/18 concentration.

reports of synergism between reversers,[20–22] but are consistent rather with the reports of the denial of such synergy.[23,24]

The data presented in this paper show that all of the 18 reversers used in Figures 2–4 are, at least, *additive* in their effects on the MDR pump. Thus, in principle, we might expect that at least some of them might be able to be used in combination chemotherapy in the clinic. What are the arguments for and against such use? Each of the reversers used in this study and listed in Table 1 is a well-studied pharmaceutical agent, with a well-characterized pharmacological effect, known dosage regime and known contra-indications.[11] They act, however, on a wide range of bodily processes (Table 1). Each interacts with its own primary receptor, but all (from the data reported here and from those of many previous investigators[1–6]) share the ability to block the MDR pump. Thus they bring about an increased cellular concentration of cytotoxic drugs and hence aid in cell killing. Figure 4 shows that, in the absence of vinblastine, each drug separately is marginally toxic to the cells at the concentration used and the combination of 10 drugs, each at 1/10 concentration, is equally toxic. Whether such an effect would be found *in vivo* (where each modulator will have an effect on a different pharmacological receptor) cannot be evaluated except in direct *in vivo* tests.

At least five of the modulators that we used in these combinations (verapamil,[12] quinidine,[13] progesterone,[25] tamoxifen[26] and trifluoperazine[27]) have been used in clinical trials of their effectiveness as reversers of the MDR pump and hence as reversers of cytotoxic drug resistance. It has been determined what levels of these reversers can be safely tolerated in the patient. These levels are also listed in Table 1 (together with the maximum tolerated plasma levels of most of the other drugs, as found by a literature search). When each of these five reversers is present on its own, the drug is effective only when it is at a concentration above (or, for quinidine, at) the tolerated level. However, when used *in combination* within our set of 10 or 18 reversers, four of the five drugs are effective at concentrations well below the tolerated level. A consideration of Table 1 shows that for eight of these reversers (amiodarone, dipyridamole, mefloquine, progesterone, quinidine, tamoxifen, terfenadine and verapamil), the tolerated plasma drug concentration is more than 10% of the value of K_i, the half-inhibition constant listed in Table 1. This means that at the tolerated level, the pump is at least 10% inhibited. However, we have shown that these drugs act together, at least additively. This means

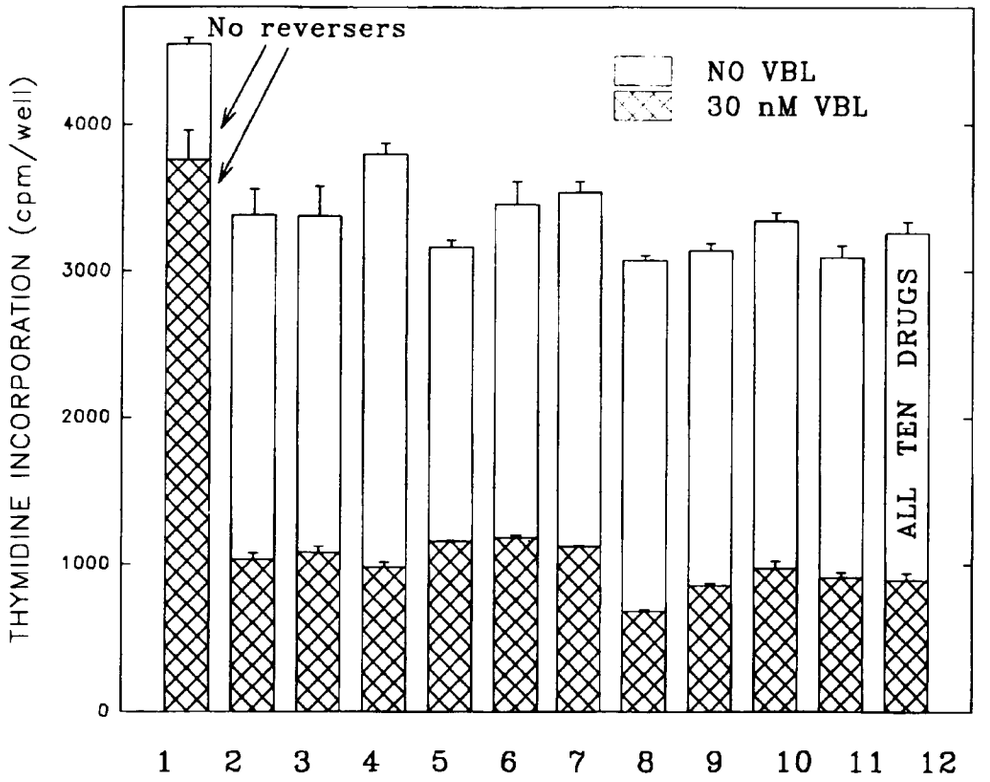

Figure 4. Effect of 10 reversers, separately and together, on inhibition of cell growth by vinblastine. MDR P388 lymphoma cells were incubated for 22 h at 37°C with (hatched bars) or without (open bars) 30 nM vinblastine, containing no reverser or the following concentrations of reversers, and then labeled with [^{3}H]thymidine and its incorporation measured. Column 1 is without any reverser; columns 2–11 are for the following reversers (concentration in parentheses in μM): 2, verapamil (6); 3, quinidine (12); 4, amiodarone (3); 5, terfenadaine (6); 6, promethazine (28); 7, reserpine (3); 8, chlorpromazine (12); 9, trifluprom azine (8); 10, mefloquine (7); 11, fluphenazine (7); column 12 is for all 10 reversers together, each at 1/10 the concentration just listed.

that if all are present together, each at their tolerated plasma level, the pump will be nearly 90% inhibited. This reasoning depends on the various modulators all having *competitive* effects against one another and, indeed, this is what our data show. There have been reports of non-competitive interaction between inhibitors of the P-glycoprotein, as measured by effects on the isolated protein[28–29] and also of competitive interactions,[30] but our data refer to many modulators acting together in the whole cell system where we have not been able to demonstrate any non-competitive effects (but see S Ayesh *et al.*, manuscript submitted).

Conclusions

Our conclusion is, therefore, that we can expect to find, in future work along these lines, combinations of a number of such drugs that, together, will reverse drug resistance as brought about by the MDR pump, yet will be tolerated by the patient. We are well aware of the fact that such drugs may interact with one another in the organism to give an unwanted response, that each has its own side-effects and that using them in combination may aggravate such a situation. Our data show, however, that these drugs act in an additive fashion so that, *with enough of them used in well-chosen combination*, each may be present at a very low concentration, insufficient to evoke any side-effects or any unwanted drug–drug interactions. Our data suggest that a low-dose multidrug approach to saturation reversal of the multidrug pump is, in principle, feasible, but the development of an effective regime of such combination reversal therapy will require detailed testing in a whole organism situation. The development of specifically designed, high effectivity, second generation modulators, such as PSC 833 (Sandoz) or GG 918 (Glaxo), will probably turn out to be

more successful but our low-dose combination approach establishes a principle that might yet be found to have clinical application, for instance, using these second generation reversers themselves in combination.

Acknowledgments

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References

1. Endicott JA, Ling V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu Rev Biochem* 1989; **58**: 137–71.
2. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* 1993; **62**: 385–427.
3. Dano K. Active outward transport of daunomycin in resistant Ehrlich ascites tumor cells. *Biochim Biophys Acta* 1973; **323**: 466–83.
4. Lehnert M. Reversal of P-glycoprotein-associated multidrug resistance: the challenge continues. *Eur J Cancer* 1993; **29**: 636–8.
5. Sikic BI. Modulation of multidrug resistance—at the threshold. *J Clin Oncol* 1993; **11**: 1629–35.
6. Lum BL, Fisher GA, Brophy NA, *et al.* Clinical trials of modulation of multidrug resistance. Pharmacokinetic and pharmocodynamic considerations. *Cancer* 1993; **72**: 3502–14.
7. Sarkadi B, Price EM, Boucher RC, *et al.* Expression of the human multidrug resistance cDNA in insect cells generates a high activity drug-stimulated membrane ATPase. *J Biol Chem* 1992; **267**: 4854–8.
8. Al-Shawi MK, Senior AE. Characterization of the adenosine triphosphatase activity of Chinese hamster P-glycoprotein. *J Biol Chem* 1993; **268**: 4197–206.
9. Ambudkar SH, Lelong IH, Zhang J, *et al.* Partial purification and reconstitution of the human multidrug-resistance pump: characterization of the drug-stimulatable ATP hydrolysis. *Proc Natl Acad Sci USA* 1992; **89**: 8472–76.
10. Shapiro AB, Ling V. ATPase activity of purified and reconstituted P-glycoprotein from Chinese hamster ovary cells. *J Biol Chem* 1994; **269**: 3745–54.
11. Reynolds REF. *Martindale—the extra pharmacopeia*, 30th edn. London: Pharmaceutical Press 1993.
12. Miller TP, Grogan TM, Dalton WS, *et al.* P-glycoprotein expression in malignant lymphoma and reversal of clinical drug resistance with chemotherapy plus high-dose verapamil. *J Clin Oncol* 1991; **9**: 17–24.
13. Wishart GC, Plumb JA, Morrison JG, *et al.* Adequate tumour quinidine levels for multidrug resistance modulation can be achieved *in vivo*. *Eur J Cancer* 1992; **28**: 28–31.
14. List AF, Spier C, Greer J, *et al.* Phase-I/II trial of cyclosporine as a chemotherapy-resistance modifier in acute leukemia. *J Clin Oncol* 1993; **11**: 1652–60.
15. Hyafil F, Vergely C, Du Vignaud P, *et al. In vitro* and *in vivo* reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative. *Cancer Res* 1993; **53**: 4595–602.
16. Johnson R, Chitnis M, Embrey W, *et al. In vivo* characteristics of resistance and cross-resistance of an adriamycin-resistant subline of P388 leukemia. *Cancer Treat Rep* 1978; **62**: 1535–47.
17. Inaba M, Kobayashi H, Sakurai Y, *et al.* Active efflux of daunorubicin and adriamycin in sensitive and resistant sublines of P388 leukemia. *Cancer Res* 1979; **39**: 2200–3.
18. Ramu A, Glaubiger D, Fuks Z. Reversal of acquired resistance to doxorubicin in P388 murine leukemia cells by tamoxifen and other triparanol analogues. *Cancer Res* 1984; **44** 4392–5.
19. Stein WD, Cardarelli C, Pastan I, *et al.* Kinetic evidence suggesting that the multidrug transporter differentially handles influx and efflux of its substrates. *Mol Pharmacol* 1994; **45** 763–72.
20. Hu XF, Martin TJ, Bell DR, *et al.* Combined use of cyclosporin A and verapamil in modulating multidrug resistance in human leukemia cell lines (see comments). *Cancer Res* 1990; **50**: 2953–7.
21. Osann K, Sweet P, Slater LM. Synergistic interaction of cyclosporin A and verapamil on vincristine and daunorubicin resistance in multidrug-resistant human leukemia cells *in vitro*. *Cancer Chemother Pharmacol* 1992; **30**: 152–4.
22. Lehnert M, Dalton WS, Roe D, *et al.* Synergistic inhibition by verapamil and quinine of P-glycoprotein-mediated multidrug resistance in a human myeloma cell line model. *Blood* 1991; **77**: 348–54.
23. Samuels BL, Ratain MJ. Correspondence re: Xiu F. Hu *et al.*, Combined use of cyclosporin A and verapamil in modulating multidrug resistance in human leukemia cell lines. *Cancer Res* 1990; **50**: 2953–7 (letter; comment); *Cancer Res* 1991; **51**: 1749–50.
24. Broxterman HF, Pinedo HM, Schuurhuis GJ, *et al.* Cyclosporin A and verapamil have different effects on energy metabolism in multidrug-resistant tumour cells. *Br J Cancer* 1990; **62**: 85–8.
25. Christen RD, McClay EF, Plaxe SC, *et al.* Phase I/pharmacokinetic study of high-dose progesterone and doxorubicin. *J Clin Oncol* 1993; **11**: 2417–26.
26. Trump DL, Smith DC, Ellis PG, *et al.* High-dose oral tamoxifen, a potential multidrug-resistance-reversal agent: phase I trial in combination with vinblastine. *J Natl Cancer Inst* 1992; **84**: 1811–16.
27. Miller RL, Bukowski RM, Budd GT, *et al.* Clinical modulation of doxorubicin resistance by the calmodulin-inhibitor, trifluoperaxine: a phase I/II trial. *J Clin Oncol* 1988; **6**: 880–8.
28. Bruggemann EP, Currier SJ, Gottesman MM, *et al.* Characterization of the azidopine and vinblastine binding site of P-glycoprotein. *J Biol Chem* 1992; **267**: 21020–6.
29. Tamai I, Safa AR. Azidopine noncompetitively interacts with vinblastine and cyclosporin A binding to P-glycoprotein in multidrug resistant cells. *J Biol Chem* 1991; **266**: 16796–800.
30. Rao US, Scarborough GA. Dirrrect demonstration of high affinity interactions of immunosuppressant drugs with the drug binding site of the human P-glycoprotein. *Mol Pharmacol* 1994; **45**: 773–6.
31. Latini R, Tognoni G, Kates RE. Clinical pharmacokinetics of amiodarone. *Clin Pharmacokinet* 1984; **9**: 136–56.

32. Hutt V, Theodor R, Pabst G, *et al.* (Plasma and urine kinetics of amitriptyline oxide and its metabolites. Comparison of intravenous infusion and oral administration in volunteers). *Arzneimittelforschung* 1991; **41**: 367–72.
33. Dahl SG. Plasma level monitoring of antipsychotic drugs. Clinical utility. *Clin Pharmacokinet* 1986; **11**: 36–61.
34. Wiesfeld AC, Remme WJ, Look MP, *et al.* Acute hemodynamic and electrophysiologic effects and safety of high-dose intravenous diltiazem in patients receiving metoprolol. *Am J Cardiol* 1992; **70**: 997–1003.
35. Dresse A, Chevolet C, Delapierre D, *et al.* Pharmacokinetics of oral dipyridamole (Persantine) and its effect on platelet adenosine uptake in man. *Eur J Clin Pharmacol* 1982; **23**: 229–34.
36. Midha KK, Marder SR, Jaworski TJ, *et al* Clinical perspectives of some neuroleptics through development and application of their assays. *Ther Drug Monit* 1993; **15**: 179–89.
37. Karbwang J, White NJ. Clinical pharmacokinetics of mefloquine. *Clin Pharmacokinet* 1990; **19**: 264–79.
38. Patel RB, Welling NJ. High-pressure liquid chromatographic determination of promethazine plasma levels in the dog after oral, intramuscular, and intravenous dosage. *J Pharmaceut Sci* 1982; **71**: 529–32.
39. Valdivieso A, Calvo R, Suarez E, *et al.* Propranolol absorption in peptic ulcer disease. *Scand J Gastroenterol* 1993; **28**: 643–6.
40. Maass AR. Studies on absorption, excretion, and metabolism of ^{3}H reserpine in man. *Clin Pharm Ther* **10**: 366–71.
41. Gardiner P, Schrode K, Quinlan D, *et al.* Spironolactone metabolism: steady-state serum levels of the sulfur-containing metabolitttes. *J Clin Pharmacol* 1989; **29**: 342–47.
42. Shall L, Newcombe RG, Lush M, *et al.* Dose–response relationship between objective measures of histamine-induced weals and dose of terfenadine. *Acta Derm Venereol Stockh* 1991; **71**: 199–204.

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