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Effect of modulators of the multidrug resistance pump on the distribution of vinblastine in tissues of the mouse

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Vinblastine at doses ranging from 0.2 to 6 mg/kg body weight was administered i.p. to mice in the absence or presence of the drugs PSC 833, cyclosporin A, mefloquine, quinidine and dipyridamole, all compounds that modulate the multidrug resistance pump and thus increase the accumulation of this cytotoxin in drug-resistant cells in cell culture. In the absence of modulators, vinblastine accumulated in tissues to different extents—lowest in brain, highest in pancreas and intestine. The extent of accumulation was directly proportional to the vinblastine dose in the range 0.2–6 mg/kg body weight. Both at high and low vinblastine doses, all the modulators except quinidine increased the ability of liver, kidney, intestine and lung to accumulate vinblastine by up to 5-fold, and with the further exception of mefloquine, also increased vinblastine levels in pancreas. Only dipyridamole had a marked effect also in brain. Cyclosporin A provided effective increases in the tissue distribution of vinblastine at plasma concentrations similar to those needed to block the multidrug pump in the case of cells in cell culture. For mefloquine, plasma concentrations three or four times higher were needed *in vivo* than were found to be effective in cell culture. The mouse system provides a quick and reliable *in vivo* method to assay modulators before they are tested in the clinic.

Key words: Cyclosporin A, dipyridamole, mefloquine, multidrug resistance, P-glycoprotein, PSC833, vinblastine.

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 cells taken from 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 protocol 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 numerous unrelated compounds, many of them being well-studied pharmaceuticals in clinical use for conditions other than cancer.[11] Some of these so-called modulators, such as verapamil,[12] quinidine,[13] cyclosporin A[14] and prochlorperazine,[15,16] have already been used in phase I and phase 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 the MDR.[17] We have followed another approach, in which combinations of known reversers can be used in an attempt to use the low specificity of the MDR pump to block it.[18,19] For this approach to be feasible, we needed to study the effects of modulators and modulators in combination *in vivo*, before moving on to human subjects. Schinkel *et al.*[20] have shown that mice which are homozygous for a defective *mdr 1a* gene ('MDR knock-out mice') show marked increases in the distribution of the cytotoxin vinblastine in many body tissues when compared with normal mice as controls. We reasoned that we should be able to see such increases in tissue distribution of vinblastine when we treated mice with known modulators of the MDR pump. We could then use this mouse assay to test the effectiveness of modulators and of combinations of modulators *in vivo* and also identify any possible deleterious interactive effects of these modulators. In the present paper, we show that

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the MDR pump modulators PSC 833, cyclosporin A, mefloquine and dipyridamole all markedly increase vinblastine levels in several tissues of the mouse. They do not all have the same effect on all tissues. Cyclosporin A is effective at plasma levels comparable to those needed to reverse the drug efflux pump in cells in cell culture.

Materials and Methods

Chemicals

Vinblastine, dipyridamole, quinidine, bovine serum albumen fraction V and sodium dodecyl sulfate were obtained from Sigma (Petah Tikvah, Israel). Mefloquine was a gift from Dr H Ginsburg, while cyclosporin A was bought from Sandoz (Basle, Switzerland) or was a gift from Dr E Shohami, and PSC833 was a gift from Dr SE Bates. Tritium-labeled compounds were: [G-^{3}H]vinblastine (52 Ci/mmol) from Rotem (Israel) and [G-^{3}H]vinblastine (21 Ci/mmol) and [G-^{3}H]daunomycin (3.9 Ci/mmol) from NEN-DuPont (Boston, MA).

Animal studies

Mice of the Sabra (Israel) strain were housed in the animal rooms of the Silberman Institute of Life Sciences, and kept in standard conditions of 12 h light and 12 h dark at 18 to 21°C. Males or females weighing 30–45 g were fed *ad libitum* until 16 h before an experiment, from which time they received only water. Mice were injected i.p. with 100 or 300 μl of vinblastine at various concentrations to which labeled vinblastine was added at 2.5 μCi per mouse. Vinblastine doses ranged from 0.2 to 6 mg/kg body weight in different experiments, but was mostly used at 0.2 mg/kg body weight. The vinblastine was dissolved in phosphate buffered saline (PBS). The modulators (dissolved in 100 μl alcohol) were given as an injection immediately after the labeled vinblastine. Control animals received only the 100 μl of alcohol together with the labeled vinblastine. After periods of time ranging from 4 to 72 h (but mostly 15 h), 1.5 ml blood was removed from the femoral artery into a tube containing sufficient EDTA to yield a final concentration of 5 mM. The mice were killed by cervical rupture and the body tissues dissected out, samples weighed and taken into homogenization buffer which was PBS supplemented with 4% bovine serum albumin fraction V, 5 mM EDTA and 0.2% sodium dodecyl sulfate, to give a final volume of 1 ml total. Homogenization was performed by sonication for 20 s for brain, liver, lung, kidney and pancreas, and 60 s for stomach, intestine and colon, using the ultrasonic processor (W-385; Heat Systems Utrasonics, New York, NY) at 76 W. After sonication, triplicate aliquots were taken for scintillation counting, as 0.25 ml into 4 ml of Quicksafe A scintillator (Zinsser Analytic, Maidenhead, UK). Aliquots of the injected vinblastine were taken for counting in order to calculate the specific activity of the injected material, and the results reported as ng or μg vinblastine per g wet weight tissue.

High performance liquid chromatography

Cyclosporin A. For the mobile phase in chromatography, acetonitrile (HPLC grade, Merck, Darmstadt, Germany) and water ('for chromatography', Merck) were used. For the extraction, diethyl ether of analytical grade was used. Standard solutions of cyclosporin A at a concentration of 2.5 μg/ml in ethyl alcohol were stored at 4°C.

Glass centrifuge tubes (15 ml) were rinsed with diethyl ether and dried. Between 200 and 400 μl of mouse plasma was added and then 4 ml of diethyl ether. The tubes were shaken mechanically for 20 min on a horizontal shaker (Lab-line Instruments, Melrose Park, IL) at 350 r.p.m. and centrifuged for 40 min at 800 *g*. A 3.2 ml aliquot of the separated diethyl ether layer was then transferred to another glass tube, which had been rinsed previously with diethyl ether. The diethyl ether extract was evaporated to dryness in a water bath, starting at 37°C and ending at 50°C. The plasma residue, concentrated now at the bottom of the glass tubes, was dissolved in 200 μl of acetonitrile and mixed on a vortex mixer. An aliquot of 100 μl was injected into the chromatograph.

The HPLC system used consisted of the Merck-Hitachi Model L-6200 intelligent pump, T-6300 column thermostat, L-3000 multi-channel photo detector and D-6000 HPLC manager. The analytical column was a Merck Lichrospher 100 RP-18, 5 μM particle size (250 × 4 mm I.D.) and a precolumn with LiChrospher 100 RP-18, 5 μM particle size (4 × 4 mm I.D).

The separations were obtained by gradient elution, using eluents A and B (A = 100% acetonitrile, B = 100% water) according to the following profile: 0–2 min, 60% A, 40% B; 2–13 min, 60–85% A, 40–15% B; 15–24 min, 60% A, 40% B. A flow rate of

1 ml/min was used. The running temperature of the column was 72°C. Calibration curves were made using cyclosporin A that had been added in defined aliquots to fetal calf serum to mimic the serum background from the mice.

Mefloquine. Acetonitrile (HPLC grade), water (for chromatography), potassium dihydrogen phosphate (GR) and orthophosphoric acid (85%, GR) were bought from Merck. Mefloquine was a kind gift from Dr H Ginsburg.

Phosphate buffer, 0.1 M, pH 3.0 was used. A 13.66 g amount of potassium dihyrogen phosphate was dissolved in about 800 ml of water and adjusted to pH 3.0 with 1 M orthophosphoric acid; after filtering through 0.2 μM disposable filter holders (Schleicher & Schuell, Germany), the solution was diluted to 1000 ml with water.

A 100 μl volume of plasma and 300 μl volume of methanol were added to microtubes of polypropylene and centrifuged in a Beckman model 11 microfuge at maximum speed for 5 min after being mixed gently on the Vortex-genie for 20 s. A 20 μl aliquot of supernatant was injected into the chromatograph.

The system was as for cyclosporin above. The analytical column was a Merck LiChrospher 100 RP-18, 5 μM particle size (250 × 4 mm I.D.) and a precolumn with LiChrospher 100 RP-18, 5 μM particle size (4 × 4 mm I.D.).

The separations were carried out using the mobile phase, consisting of acetonitrile: 0.1 M phosphate buffer (pH 3.0) (35:65, v/v) at a flow rate of 1.5 ml/min.

Results

Figure 1 depicts the time course of disappearance of labeled vinblastine from four tissues of mice that were injected with vinblastine, in the absence of any modulators, at 6 mg/kg body weight. The drug is taken up into the tissues before the first samples are taken (4 h) and lost exponentially with a half-time of the order of 5–8 h in the different tissues. For most of the subsequent experiments, a standard time of 16 h was chosen since this gave satisfactorily high tissues levels of drug and, from the comparable experiments of Schinkel *et al.*[20] with knock-out mice, would be expected to show a large difference between modulator-treated and control mice. Figure 2 shows, for four tissues, the amount of drug still present in the tissues at 16 h, in the absence of modulators, as a function of the concentration of the unlabeled vinblastine administered together with the label. The accumulation of vinblastine in the tissues seems to be a linear function of the dose, suggesting that intracellular receptors for vinblastine do not become saturated with increasing tissue concentrations of the drug in the concentration range studied. The concentration of vinblastine measured in the blood at 16 h was 12 nM when the dose administered was 0.2 mg/kg body weight and 800 nM when 6 mg/g was administered.

We performed many control experiments in which vinblastine at 200 μg/kg body weight was administered to mice without any addition of a putative modulator of the multidrug pump and the tissue distribution of the drug was measured 15 h later. Table 1 records these results for the eight tissues that we studied in detail, as the means (in ng vinblastine per g wet weight of tissue) ± SD. Brain seems to take up the least drug, as measured in these units, pancreas the most. The data for colon and intestine showed the most scatter between individual determinations, brain the least.

Figure 3 depicts the effects of a simultaneous injection of the non-immunosuppressive MDR pump reverser PSC833 at 15 mg/kg body weight

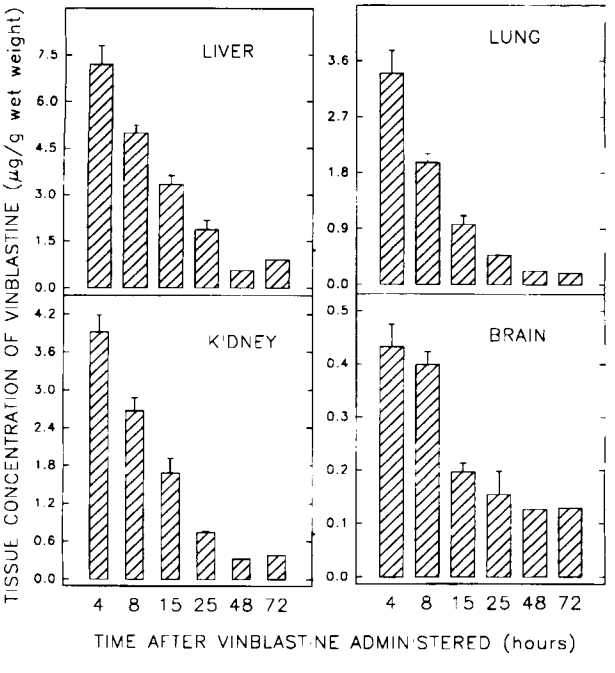

Figure 1. Time course of disappearance of labeled vinblastine from tissues of the mouse. Vinblastine dose was 6 mg/kg body weight, i.p. Triplicate determinations at 4 and 8 h, eight determinations at 15 h, duplicates at 25 h, and singles at 48 and 72 h. Data are depicted as means ± SE, where applicable (spread at $n = 2$). Half-times of disappearance of label (fitting to an exponential decay curve) were 8.1, 5.1, 7.3 and 7.0 h for liver, lung, kidney and brain, respectively.

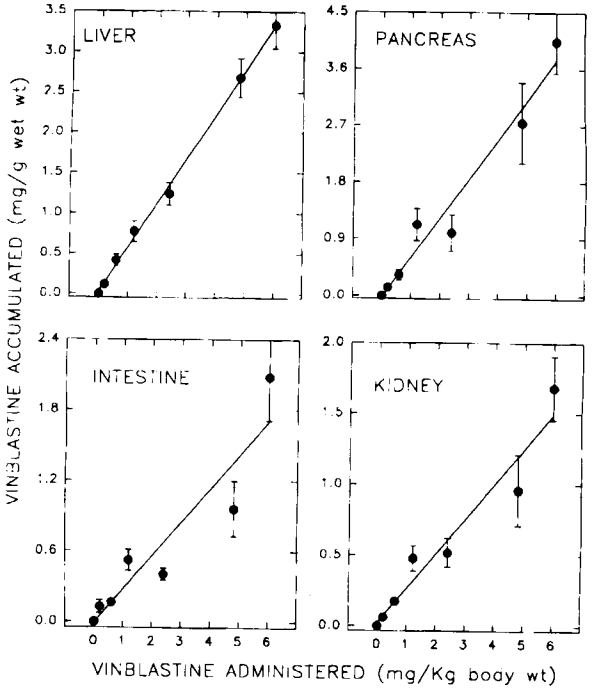

Figure 2. Dependence of vinblastine accumulation in four tissues of the mice as a function of the dose of vinblastine, administered i.p. Mice were killed at 15 h and tissues dissected. Data are depicted as means ± SE. The number of determinations was 17 at 0.2 mg/kg, three at 0.6, 1.2, 2.4 and 4.8 mg/kg, and six to 10 for the different tissues at 6 mg/kg body weight.

Table 1. Vinblastine uptake in male mice (about 45 g weight) injected with ^{3}H-labeled cytotoxin at 0.2 mg/kg body weight and killed 15 h later.

	Mean content of Vinblastine (± SD) (ng/g wet wt)	No. of observations
Brain	6.0 ± 0.3	5
Lung	48 ± 24	17
Liver	116 ± 30	17
Pancreas	132 ± 59	17
Stomach	66 ± 18	5
Intestine	128 ± 74	17
Colon	100 ± 75	17
Kidney	62 ± 22	20

mouse, together with vinblastine at 6 mg/kg body weight, on the tissue levels of vinblastine, measured at 15 and 48 h after drug administration. The effects on brain are here small, whereas the effects of PSC833 on the accumulation of vinblastine in kidney, lung and also liver are striking. Figure 4 shows a more detailed study using the immunosuppressor cyclosporin A as the putative modulator of the MDR pump. Increasing concentrations of cyclosporin (from 0 through 100 mg/kg body weight) were

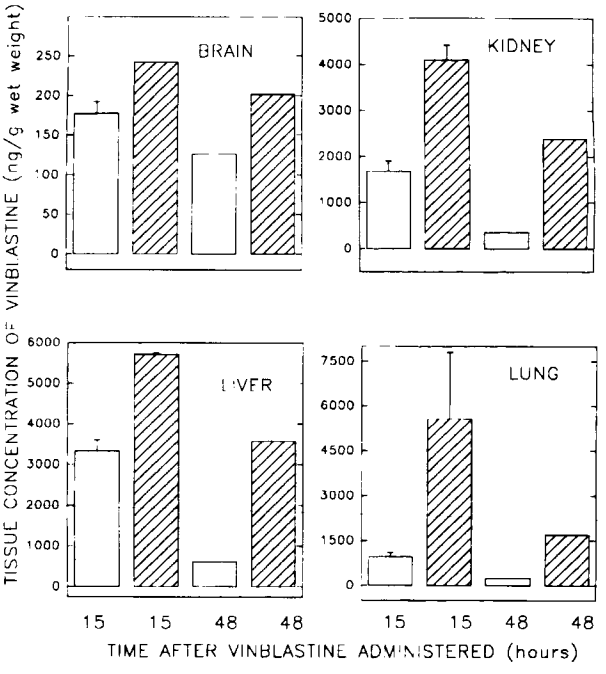

Figure 3. Effect of the modulator PSC833 at 15 mg/kg body weight on the accumulation of vinblastine (6 mg/kg) in four tissues of the mice. Mice killed at 15 or 48 h after injection of vinblastine together with PSC833. Duplicate determinations at 15 h (spread depicted), singles at 48 h. Hatched bars are with PSC833, open bars are controls.

administered together with the vinblastine (at 200 μg/kg body weight) and the tissue distribution of vinblastine measured after 15 h. In five tissues (intestine, lung, liver, pancreas and kidney—all rich in P-glycoprotein[21]), tissue levels of vinblastine increased proportionately with increasing concentration of cyclosporin administered, with saturation of the effect being achieved by 100 mg cyclosporin A/kg body weight. Again, as with PSC833, the effects on brain were small (data not shown). Vinblastine accumulation in colon did not seem to be affected by increases in the modulator concentration above 25 mg/kg body weight.

Figure 5(A) shows levels of vinblastine present in kidney tissue 15 h after the mice were given vinblastine at 200 μg/kg body weight and various concentrations of cyclosporin A. In these experiments, the concentration of cyclosporin A was measured, using HPLC, in serum taken from the mice at sacrifice. The vinblastine accumulation is here plotted against the *measured* concentration of the modulator. Each point depicts values from two to four different mice, with the data from mice having comparable measured plasma levels being lumped together. Accumulation appears to saturate at increasing plasma levels of cyclosporin A. The increase is half-

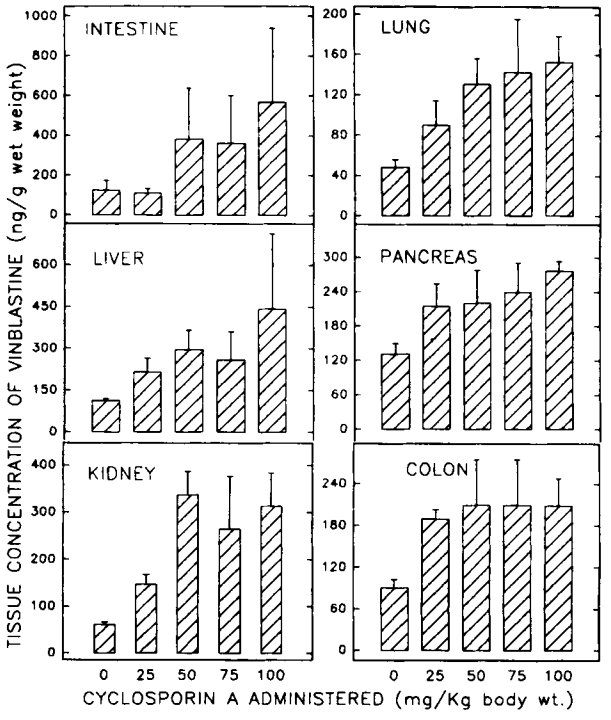

Figure 4. Effect of increasing doses of cyclosporin A on accumulation of vinblastine (at 0.2 mg/kg body weight), measured 15 h after administration of the drugs together, i.p. The number of determinations was 17 at zero cyclosporin A, two at 25 and 100 mg/kg, and three at 50 and 75 mg/kg body weight. Data are given as means ± SE or spread if $n = 2$.

maximal at a serum concentration of 3.3 (± 1.7) μM and reaches a maximum of some 6.5 times the control level. Similar data were found (not shown) when the lungs and livers of these mice were assayed and, in a less comprehensive set of experiments (data not shown), for pancreas, intestine and colon. Figure 5(B) presents data similar to those in Figure 5(A), but depicting the levels of labeled vinblastine in the plasma of these mice. Apparently, treatment of mice with cyclosporin A increases the level of vinblastine in the plasma, as well as increasing accumulation in the tissues. (Similarly, Schinkel *et al.*[20] found for mice homozygous for a knocked-out *mdr1a* gene, that plasma levels of vinblastine increased 2- to 5-fold as compared with the wild-type mice.) The plasma concentration of label in Figure 5(B) increases linearly and doubles over this concentration of cyclosporin A, but the increases in drug accumulation in the tissues are considerably greater.

In order to separate out the effects of cyclosporin A on plasma levels of vinblastine from its effects on tissue levels, we had to take into account the plasma level increase. To do this, we proceeded as follows. The data on plasma levels of labeled vinblastine were first fitted by linear regression yielding the equation $y = 1.08 + 0.065 \times C$, where C is the concentration of cyclosporin A. (Attempts to fit the data of Figure 5(B) to an equation compatible with saturation of cyclosporin's effect were not successful, the linear fit being demanded by the data). With this regression equation, we could then calculate the plasma level of vinblastine at each different plasma level of cyclosporin. Using these *computed* plasma levels, we could then calculate the degree to which the vinblastine was further concentrated in the tissue over and above its concentration in plasma. We term this the *relative* vinblastine accumulation.

The results of such computations for liver, lung and kidney are presented in Figure 6. For each tissue, we plot the ratio of vinblastine accumulated in the tissue, at a given concentration of cyclosporin A, to the amount accumulated in the absence of cyclosporin A, divided by the degree to which the plasma concentration of vinblastine is increased over controls by the same concentration of cyclosporin A. The effect of cyclosporin A as modulator appears to saturate with its concentration. Half-saturation is found at cyclosporin A concentrations of 1200, 500 and 800 nM, for liver, lung and kidney, respectively.

In order to compare effects of cyclosporin A in mice *in vivo* with its effects on cells in cell culture, we performed experiments, following Lan *et al.*[18] in which P388 leukemia cells, resistant to adriamycin, were allowed to accumulate labeled vinblastine at 2 nM during 40 min at room temperature, in the presence of increasing concentrations of cyclosporin A. We performed experiments both in our conventional accumulation medium[18,19] containing 10% fetal calf serum, and in a medium containing 100% fetal calf serum, in order to more closely simulate the conditions in the living animal. In both cases (data not shown, but see Lan *et al.*[18]), the concentration of cyclosporin A that gave half-maximal saturation of vinblastine accumulation was about 280 nM, comparable to the level at which it gives a half-maximal stimulation in the mice *in vivo*.

We studied the effects of three other drugs on the tissue distribution of vinblastine. Figure 7 depicts data obtained using the antimalarial mefloquine, administered i.p. together with the vinblastine (at 200 μg/kg body weight). Mefloquine was given here at doses ranging from 5 to 100 mg/kg body weight (Table 2), and the resulting plasma level of the modulator assayed by HPLC.[22] The increases in tissue vinblastine levels were striking for intestine

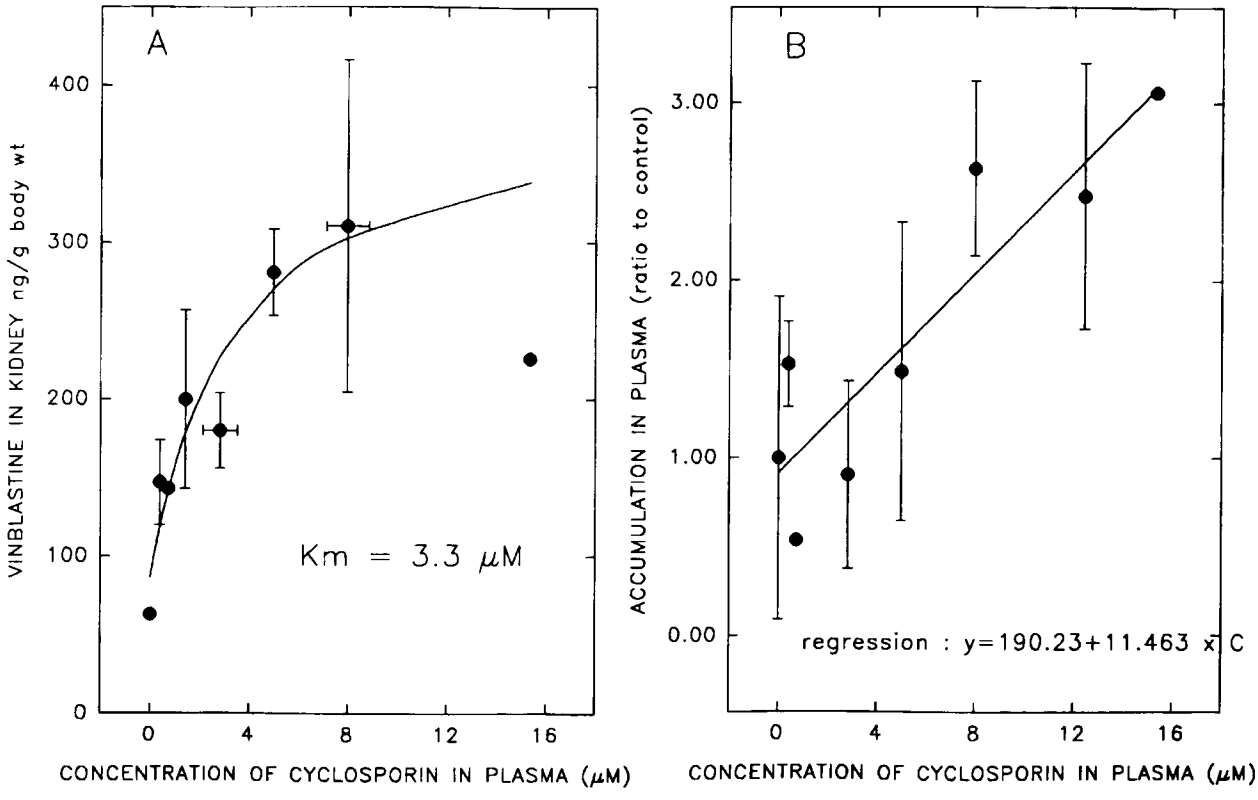

Figure 5. Effect of increasing plasma concentrations of cyclosporin A on accumulation of vinblastine (at 0.2 mg/kg body weight), measured 15 h after administration of the drugs together, i.p. (A) Accumulation in kidney, (B) level in plasma as ratio to control. Cyclosporin A concentration determined by HPLC for each mouse and the data at close concentrations lumped. Bars show ± SE (or spread) in both the *x* and *y* axes. Two or three mice at each data point. The line in (A) is the best-fit according to the Michaelis equation $y = V_0 + V_{max} \times (C/(C + K_m)$, where *C* is the plasma concentration of cyclosporin A, V_0 the vinblastine accumulation at zero cyclosporin, V_{max} the maximal increment in accumulation and K_m is the cyclosporin concentration at which one-half the maximal increment is found. Here, $K_m = 3.3$ μM. In (B) the line is the linear regression with $y = 1.08 + 0.0651 \times$ [cyclosporin A]. In each case, the vinblastine accumulation datum and the corresponding blood plasma level of vinblastine come from the same mouse.

and kidney, but less so for lung and liver, and hardly noticeable, if at all, for pancreas and colon. The level in brain was little affected. Similar but less detailed results (not shown) were found using 6 mg/kg vinblastine.

Figure 8 depicts data obtained for the effect of the anticoagulant dipyridamole, injected i.p. in doses up to 50 mg/kg body weight, on tissue level of vinblastine, measured 15 h after the two drugs were administered simultaneously.[23] Again, tissue levels are strikingly increased, but here levels in brain are also affected significantly. Finally, we studied the effects of quinidine injected at doses up to 100 mg/kg body weight. We found no increase in the accumulation of labeled vinblastine in any of the tissues we assayed (lung, liver, pancreas, kidney, colon and kidney) when we measured this modulator's effect after 15 h. Since we reasoned that the quinidine might no longer be present in the body after 15 h,[6] we also studied the effect of quinidine at 6 and 4 h after injection together with vinblastine. We found no effect of the quinidine (data not shown for all these studies).

Discussion

Our studies were designed to test whether known modulators of the MDR pump would have the same effect in increasing vinblastine accumulation in tissues as has been found for mice knocked out in their *mdr1a* gene.[20] Have our studies confirmed this hypothesis? First, our data and those of Schinkel *et al.*[20] demonstrate a rather similar picture in the controls (mice homozygous for an intact *mdr1a* gene and mice not given modulators, respectively), although our determinations have been made using radiolabeled vinblastine, theirs by measuring vin-

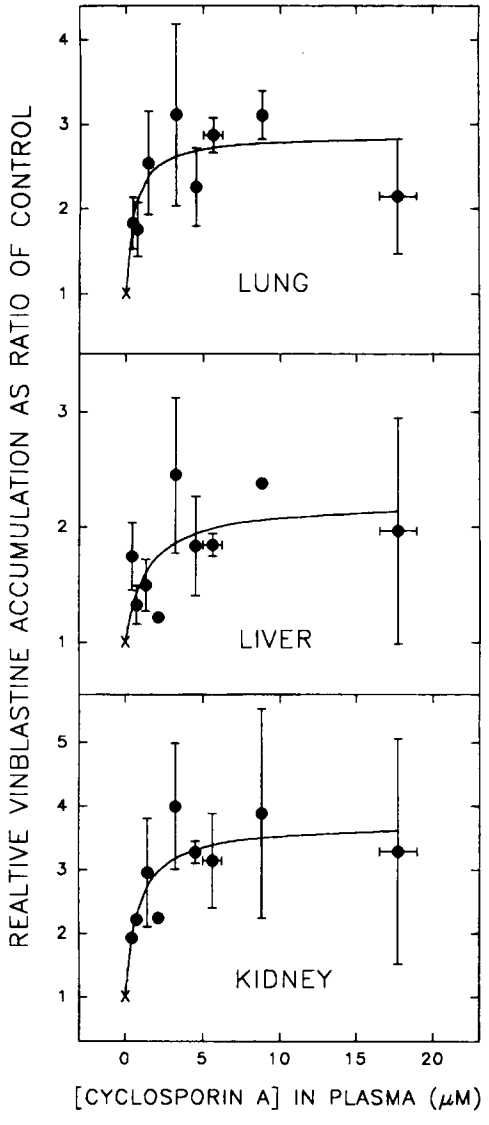

Figure 6. The ratio of accumulation of vinblastine in three tissues relative to controls, corrected for the concomitant increase in blood plasma vinblastine, plotted against [cyclosporin] in plasma, determined by HPLC. The values of the ratio of vinblastine accumulated in a tissue, relative to control values, i.e. data such as depicted for kidney in Figure 5(A), were divided by the calculated incremental ratio of blood plasma vinblastine (calculated from the regression in Figure 5(B) to give the relative vinblastine accumulation as compared with the plasma level. These were plotted on the abscissa against [cyclosporin] as ordinate. The lines are fitted to the Michaelis equation as in Figure 5(A), except that the relative accumulation at zero cyclosporin was set to be unity.

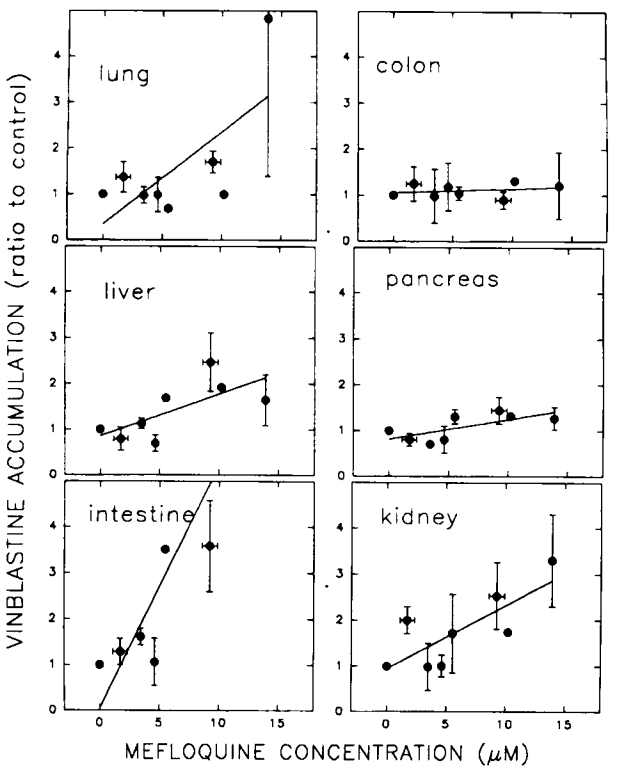

Figure 7. Effect of increasing plasma concentrations of mefloquine on accumulation of vinblastine (at 0.2 mg/kg body weight), measured 15 h after administration of the drugs together, i.p. Mefloquine concentration determined by HPLC for each mouse and the data at close concentrations lumped. Bars show ± SE in both the *x* and *y* axes. Line is regression through the individual data points. Correlation coefficients are 0.695, 0.264, 0.701, 0.700, 0.933 and 0.783 for data sets for lung, colon, liver, pancreas, intestine and kidney with 13 degrees of freedom.

blastine using HPLC. We both find, for example, that vinblastine is rapidly accumulated in body tissues of the mouse and subsequently leaves those tissues with half-lives of some 5–8 h (our Figure 1, their Table 3), varying somewhat from tissue to tissue. Table 1, which records data on the accumulation of vinblastine from a low i.p. dose, shows that brain takes up the least drug (again, as Schinkel *et al.* found), while intestine, pancreas and liver take up the most. For three tissues, i.e. brain, kidney and liver, Schinkel *et al.*[20] report values of the amount of vinblastine accumulated, 8 and 24 h after an injection of 6 mg/kg body weight, which can be directly compared with our data collected in Figure 2. Our numbers are very similar to theirs as far as kidney is concerned, but for brain and liver, our mice seem to incorporate 5–20 times more drug. Our data and theirs are strictly comparable for both kidney and lung at 4 h, but not for brain and liver, where again our numbers are an order of magnitude higher. It is not clear if these differences arise from the different strains of mice used or from the route of administration of the drug (i.p. in our case, tail vein in theirs) or from our use of vinblastine in radiolabeled form, so that our assays count both true vinblastine and its metabolic breakdown products.

Table 2. Vinblastine uptake in male mice (about 45 g weight) injected with ^{3}H-labeled cytotoxin at listed dose, with/without mefloquine, and killed 15 h later

	0.2 mg/kg (no mef)	0.6 mg/kg (no mef)	6.0 mg/kg (no mef)	20 mg/kg (mef ratios,	60 mg/kg 0.2 VBL)
Brain	6.0	18.8	193	1.3	1.1
Lung	24.6	77.4	1037	3.1	9.5
Liver	108	321	3904	1.8	1.8
Pancreas	134	422	4037	1.6	1.3
Stomach	67.6	357	1578	2.3	1
Intestine	86.5	215	2081	3.2	8
Colon	67.5	841	6288	1.6	1.8
Kidney	18.0	145	1674	19.2	11

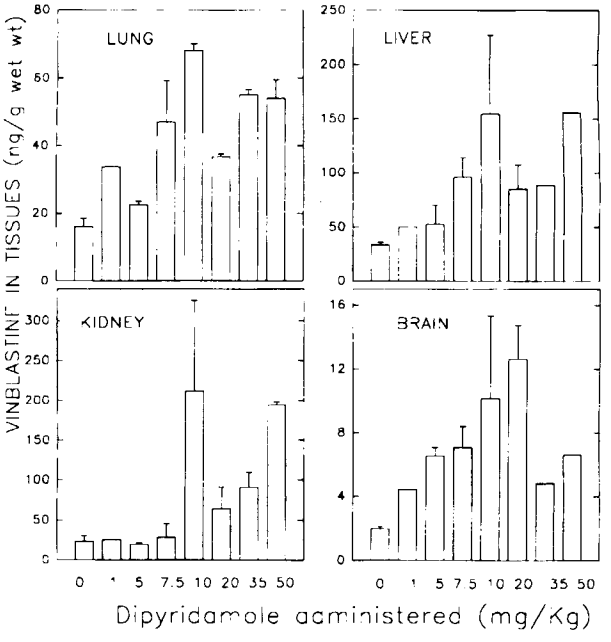

Figure 8. Effect of increasing doses of dipyridamole on accumulation of vinblastine (at 0.2 mg/kg body weight), measured 15 h after administration of the drugs together, i.p. Number of determinations was 17 at zero cyclosporin A, one at 1 mg/kg and two at the higher doses (singlicates for brain at the two highest doses). Data are given as means ± SE or spread if $n = 2$.

The amount of labeled vinblastine taken up in the various tissues appears to depend linearly on the concentration of vinblastine in the range 0.2 to 6 mg administered per kg body weight. This, too, is similar to what can be calculated from Schinkel *et al.*[20], on comparing their Tables 2 and 3, for experiments at 1 and 6 mg/kg body weight, respectively.

The experiments of Schinkel *et al.*[20] on mice containing a double knockout of the *mdr1a* gene showed that many tissues of these mice took up larger amounts of vinblastine than the control mice, ratios of 10- to 40-fold in different tissues being found. In comparable fashion, but with significant differences, our Figures 3–8 show that the amount of drug accumulated is increased by treatment of the mice with drugs known to be modulators of the MDR pump. (The drug PSC883 was specifically designed to be a reverser of this pump and is being used in clinical trials to overcome MDR)[24]. Similarly, cyclosporin A is a modulator of the pump[25] and has been used in clinical trials.[26]) We have found that mefloquine reverses the multidrug pump's action on cytotoxin accumulation in P388 leukemia cells,[18] while dipyridamole has also been shown to be an effective reverser of the multidrug pump.[18,27] For none of these can one argue that their sole effect in the body is on the multidrug pump but this is certainly one feature that they share in common.

It would appear, however, that the various tissues are not equally sensitive to the effects of the MDR pump modulators and that the different modulators have some individual tissue specificities. Thus, only intestine, liver and kidney are strongly affected by mefloquine. Brain is far less affected by PSC833 than is lung or kidney (Figure 3) and little affected by cyclosporin A or mefloquine (data not shown), but is very sensitive to the action of dipyridamole (Figure 8). It should be noted that brain was the tissue most affected by the presence of the knock-out gene for MDR2,[20] so we were surprised to find that three of our four modulators did not affect the distribution of vinblastine in brain. It may be that these drugs are not able to reach high enough concentrations at the sites in the blood–brain barrier where the P-glycoprotein exerts its action. These results have some obvious clinical implications for the use of modulators in attempts to enhance the effectiveness of chemotherapy. Those modulators which do not affect the degree of accumulation of cytotoxins in brain will be more valuable in the treatment of tumors in sites other than brain. In contrast, if the intention is to increase the concentration of cyto-

toxin in brain itself, so as to target chemotherapy there, modulators which affect brain tissue should be chosen. Tumors originating in intestine, liver or kidney might be most sensitive to modulators of the class of mefloquine.

Interestingly, the plasma levels at which cyclosporin A is active *in vivo* (Figure 6) are comparable to those at which it is active in our *in vitro* studies.[18,19] This is perhaps surprising since the effect of modulators *in vivo* are a complex function of their effects on the multidrug pump (or pumps) in the cell membrane, of the wash out of vinblastine from the body and of its metabolism. Our finding that mefloquine is less effective *in vivo* than in cell culture studies might be more expected. That quinidine is not effective at all, in our experience, is compatible with its near total lack of effect in clinical trials. In all these cases, a future task will be to explore the basis for the lack of effect of such modulators. This includes also the low effects of cyclosporin A and PSC 833 on brain.

Conclusions

Our data are compatible with the interpretation that PSC833, cyclosporin A, mefloquine and dipyridamole increase the tissue distribution of vinblastine by their effect on blocking the multidrug pump, although effects on other drug pumps such as the MRP pump[28,29] cannot be ruled out. Our data show that the effects of such modulators acting alone or in combination[18,19] and also the new, designed modulators can be readily tested in animals *in vivo* to give a firm experimental basis before clinical trials are attempted.

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