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The abamectin derivative ivermectin is a potent P-glycoprotein inhibitor

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Among the compounds endowed with the capacity to reverse the P-glycoprotein (Pgp)-mediated multidrug resistance of cancer cells, a powerful agent was found to be the cyclosporin D derivative SDZ PSC 833. After *in vivo* treatment with SDZ PSC 833, mice showed a decreased tolerability to cyclosporin A (CsA), but also to ivermectin, a widely used polycyclic lactone pesticide of *Streptomyces avermitilis* origin. The sequels were suggestive of CsA- or ivermectin-induced central nervous system dysfunction; they were interpreted as caused by the neutralization of the Pgp at the blood–brain barrier level, implying that CsA and ivermectin were Pgp substrates. CsA was already known to display both Pgp substrate and Pgp inhibitor properties. It now appears that ivermectin may also inhibit Pgp function. When compared in short-term assays for Pgp function inhibition, which measure the restoration of the retention of two Pgp probes in multidrug-resistant (MDR) cells to their parental (Par) cell levels, ivermectin appeared only a few fold weaker than SDZ PSC 833 in the case of murine monocytic leukemia MDR-P388 cells and nearly as active as SDZ PSC 833 in the case of human lymphocytic leukemia MDR-CEM cells. Therefore, like CsA or FK-506, ivermectin may be both a substrate and an inhibitor of Pgp.

Key words: Ivermectin, multidrug resistance, P-glycoprotein, SDZ PSC 833.

Introduction

P-glycoprotein (Pgp) molecules can confer multidrug resistance (MDR) to cancer cells by reducing their uptake of a variety of cytostatic and cytotoxic agents, among which are some of the most efficient anti-cancer drugs. Chemosensitization of such MDR cells to drugs which are Pgp substrates is obtained by a variety of modulators, two of the most potent of which are the cyclosporin D derivative SDZ PSC 833 and the semi-synthetic cyclopeptolide SDZ 280-446.[1]

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While performing tolerability studies in the mouse for the combination of cyclosporin A (CsA) and SDZ PSC 833 or SDZ 280-446,[2] we observed signs of potential central nervous system (CNS) toxicity suggestive of CsA-mediated neurotoxicity.[3] Pgp expression at the blood–brain barrier (BBB) level[4,5] would reduce the accumulation of toxic metabolites or blood-carried xenobiotics in the CNS. Indeed, *mdr1a knock-out* (*mdr1a*^{KO}) mice were found to have a BBB deficiency, particularly evidenced by a much increased sensitivity to Ivomec™, a commercial preparation of a widely used pesticide, ivermectin.[6] Ivermectin is a $GABA_A$ receptor agonist whose general safety of use in mammals depends on an intact BBB.

Therefore, we extended our tolerability studies to the combination of the same Pgp blockers with Ivomec™, and we found that interference with the Pgp function would dramatically reduce the ivermectin tolerability in the mouse.[2] The simplest hypothesis was thus that Pgp molecules expressed on the endothelial cells of BBB capillaries were able to efflux CsA and ivermectin, and that strong inhibition of the BBB-associated Pgp by a Pgp blocker would facilitate diffusion of CsA and ivermectin into the brain tissue. While we had presumed that ivermectin would be a fast Pgp substrate, being easily effluxed by the Pgp molecules expressed at the luminal side of CNS capillary endothelial cells, it came out that ivermectin was itself a potent inhibitor of Pgp function.

Materials and methods

Tumor cell lines and drugs

The parental (Par) and multidrug-resistant (MDR) tumor cell lines were the earlier described murine monocytic leukemia Par-P388 and MDR-P388, and human T leukemia Par-CEM and MDR-CEM.[8]

The anti-cancer drugs were doxorubicin (adriamy-

cin–HCl; Serva, Heidelberg, Germany) and vinblastine (vinblastine sulfate; Lilly, Giessen, Germany). The Pgp probes, rhodamine-123 [R-123 (MW = 380), Eastman Kodak, Rochester, NY] and daunomycin [DAU (MW = 564), Sigma, St Louis, MO] were prepared as stock solutions at 1 mg/ml, respectively, in bidistilled water and 0.9% NaCl. Their final concentrations in the culture medium were 5 μg/ml (13.16 μM) for R-123 and 20 μM (11.3 μg/ml) for DAU.

The compounds assayed for Pgp inhibition were prepared as stock solutions in absolute ethanol at 10 mM for verapamil (MW = 491, Sigma) and at 10 mg/ml (about 8 mM) for CsA (MW = 1206.6), SDZ PSC 833 (MW = 1214.65) and SDZ 280-446 (MW = 1182.6) (all from Sandoz Pharma Ltd, Basel, Switzerland). For these modulators, 1 μg/ml = 2.0 μM for verapamil, 0.8 μM for CsA, SDZ PSC 833 and SDZ 280-446. Azadirachtin A (MW = 720.23; 1 μg/ml = 1.4 μM) was provided by Sandoz and dissolved at 1 mg/ml in ethanol as a stock solution. Ivermectin was bought as Ivomec™ (Merck Sharpe and Dohme, Haarlem, The Netherlands), an injectable solution containing 10 mg/ml ivermectin [as this is a mixture of > 80% ivermectin B1α (MW = 874) and < 20% ivermectin B1β (MW = 860), 1 μg/ml = 1.14 μM, based on the 874 MW value]. For control assays, the Ivomec™ solvent was reconstituted as a 40% glycerol formal (Sigma) and 60% propylene glycol (Sigma) mixture.

In order to assay ivermectin in other solvents commonly used for modulator studies, some ivermectin was repurified by Dr E Lier (Sandoz Pharma) from Ivomec™. Bulb to bulb distillation of 2 × 50 ml bottles of commercially available Ivomec™ solution at 80–90°C/2 × 10^{-2} mBar afforded a white powder (1.146 g), m.p. 83–87°C, $[\alpha]_D$ +33.5° (0.9% in $CHCl_3$) which was recrystallized from aqueous ethanol to give a white solid (901 mg), m.p. 135–145°C, $[\alpha]_D$ +37° (0.8% in $CHCl_3$) as a mixture of isomers (about 90:10, based on NMR). This repurified ivermectin will be named IVM in all further experiments; it was found to contain 10% or less ivermectin B1β, thus half the ivermectin B1β amount of Ivomec™. For cellular studies, IVM was dissolved in DMSO.

Assays of Pgp function inhibition

Inhibition of Pgp function was studied by the restoration of MDR cell chemosensitivity to anti-cancer drugs (growth inhibition assay in culture), and by the restoration of the retention of fluorescent Pgp probes in MDR cells (fluorescence cytometry assays).

The tumor cell growth and its drug-mediated inhibition were measured by the previously described MTT assay.[8] The cytostatic IC_{50}^+ values were determined in the presence of various modulator concentrations (0, 0.1, 0.3, 1.0 and 3.0 μg/ml), while the IC_{50}^- values were obtained in the presence of the modulator solvent only. The increased cytostatic sensitivity was expressed as 'resistance decrease factors' (RDF) which were calculated by the ratio: IC_{50}^-/IC_{50}^+.[8]

The effluxing function of MDR cell Pgp molecules and its inhibition were measured by the previously described fluorescence cytometry assays performed on microplate bulk cell suspensions for the retention of DAU or R-123.[8] The results were represented as 'relative retention' of the fluorescent probes of Pgp function, i.e. MDR cell fluorescence as percentage of the fluorescence of similarly treated Par cells, measured at the various modulator concentrations;[8] a modulator EC_{50} was defined as the concentration which restored the MDR cell fluorescence to 50% of the one shown by similarly treated Par cells. The final modulator concentrations in culture medium depended on the assay, the full scale being covered by the 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10 and 30 μg/ml concentrations.

Results

Most experiments were performed with human Par-CEM and MDR-CEM cells, only control ones being done with murine Par-P388 and MDR-P388 cells. For reasons detailed earlier,[8] the CEM data may be more relevant for MDR cancer treatment, as anti-cancer drug efflux is mediated by a single *MDR1* encoded Pgp in human, whereas it may be mediated by two different *mdr1a* and *mdr1b* encoded Pgp in the mouse; the latter show different tissue expression, may confer different profiles of drug resistance and show different profiles of susceptibility to modulators. As we do not know which of *mdr1a* and/or *mdr1b* is expressed on the MDR-P388 cells, data obtained with MDR-CEM cells are definitely more relevant for human Pgp inhibition in the clinic. Most experiments focused on the capacity of ivermectin, in comparison with a few reference modulators, to increase the retention of fluorescent Pgp probes, such as R-123 or DAU, by interfering with the efflux of those typical Pgp probes. A few assays of chemosensitization of MDR cells to growth inhibition by anti-cancer drugs were also performed. In

the following text and tables, Ivomec™ represents the commercial ivermectin solution and IVM represents the repurified ivermectin.

Restoration of Pgp probe retention by MDR-CEM cells by ivermectin

When analyzed by bulk culture fluorescence spectrometry, *in vitro* cultured MDR-CEM cells showed a 11 ± 8% DAU retention and a 8 ± 3% R-123 retention in comparison to Par-CEM cells. These DAU and R-123 retention values did not significantly change in the presence of the modulator solvents (0 μg/ml modulator control values in Tables 1 and 2). The restoration of Pgp probe retention in the MDR cells, at increasing modulator concentrations, could be measured as a percentage of the similarly treated Par cells, as the modulator treatment of the latter did not change their Pgp probe retention [except for a slight decrease (≤ 20%) of R-123 retention at the highest ivermectin dosage; not shown].

The capacity of ivermectin, either the original Ivomec™ solution or the purified IVM macrolide, to restore the retention of DAU (Table 1) and R-123 (Table 2) in MDR cells could be compared with those of reference Pgp modulators, as concentrations required to restore 50% of the Par cell retention levels (EC_{50}). With DAU, the following EC_{50}s could be calculated: 0.41 μM for Ivomec™, 0.59 μM for IVM, 20.4 μM for verapamil, 1.12 μM for CsA, 0.30 μM for SDZ PSC 833 and 0.25 μM for SDZ 280-446. With R-123, the EC_{50}s were found to be 4.32 μM for Ivomec™, 6.69 μM for IVM, 4.81 μM for SDZ PSC 833 and 3.46 μM for SDZ 280-446, but could not be achieved for verapamil and CsA. Thus, the macrolide ivermectin was a very potent inhibitor of Pgp function, though less than the most potent modulators; particularly, it was a stronger modulator than CsA and verapamil.

Restoration of Pgp probe retention by MDR-P388 cells by ivermectin

When analyzed by bulk culture fluorescence spectrometry, *in vitro* cultured MDR-P388 cells showed a

Table 1. Restoration of the DAU retention in MDR-CEM cells by various modulators

Modulator (μg/ml)	Retention in MDR cells as percent of its retention in similarly treated Par cells[a]								
	0	0.01	0.03	0.1	0.3	1	3	10	30
SDZ PSC 833	13 ± 8	18 ± 9	25 ± 10	33 ± 11	44 ± 15	78 ± 18	83 ± 17	81 ± 10	98 ± 19
Ivomec™	10 ± 9	18 ± 3	22 ± 4	31 ± 13	44 ± 13	77 ± 13	84 ± 19	94 ± 10	93 ± 11
IVM	10 ± 9	12 ± 5	18 ± 5	26 ± 6	35 ± 11	67 ± 11	84 ± 17	85 ± 22	94 ± 20
SDZ 280-446	13 ± 8	17 ± 1	32 ± 6	44 ± 20	50 ± 22	71 ± 22	85 ± 23	90 ± 10	93 ± 11
CsA	13 ± 8	16 ± 6	25 ± 3	26 ± 3	26 ± 7	45 ± 16	60 ± 7	83 ± 16	112 ± 21
Verapamil	13 ± 8	19 ± 6	23 ± 6	27 ± 7	31 ± 8	38 ± 14	40 ± 14	50 ± 8	73 ± 7

[a]The results are shown as means (±SD) of three to 10 independent experiments, each assay being performed in duplicate.

Table 2. Restoration of the R-123 retention in MDR-CEM cells by various modulators

Modulator (μg/ml)	Retention in MDR cells as percent of its retention in similarly treated Par cells[a]					
	0	0.3	1	3	10	30
SDZ PSC 833	6 ± 3	9 ± 2	14 ± 8	32 ± 14	63 ± 5	84 ± 9
Ivomec™	14 ± 6	13 ± 5	20 ± 10	44 ± 16	69 ± 19	89 ± 25
IVM	12 ± 9	11 ± 3	16 ± 8	38 ± 15	59 ± 13	96 ± 17
SDZ 280-446	6 ± 3	13 ± 4	24 ± 13	45 ± 20	63 ± 13	78 ± 7
CsA	6 ± 3	6 ± 2	7 ± 2	8 ± 1	13 ± 4	31 ± 10
Verapamil	6 ± 3	10 ± 5	9 ± 3	9 ± 3	10 ± 5	12 ± 6

[a]The results are shown as means (±SD) of three or five independent experiments, each assay being performed in duplicate.

0 ± 0% DAU retention and a 7 ± 2% R-123 retention in comparison to Par-P388 cells. In the presence of the modulator solvents (0 μg/ml control values in Table 3), the DAU retention values did not change but the R-123 retention values showed some increase. The restoration of Pgp probe retention in the MDR cells at increasing modulator concentrations was reported as a percentage of the similarly treated Par cells, as the modulator treatment of the latter did not change their Pgp probe retention [except for a slight decrease (≤ 20%) of R-123 retention at the highest ivermectin dosage; not shown]. Both ivermectin preparations and SDZ PSC 833 (as reference) were compared for their capacity to restore the retention of DAU and R-123 (Table 3). The EC_{50}s for DAU retention restoration were 5.27 μM for Ivomec™, 8.50 μM for IVM and 0.13 μM for SDZ PSC 833, and those for R-123 were 5.95 μM for Ivomec™, 5.49 μM for IVM and 0.38 μM for SDZ PSC 833. Thus, with the MDR-P388 cells, ivermectin was a potent inhibitor of Pgp function, though much less active than SDZ PSC 833.

Lack of detectable inhibition of Pgp function by Azadirachtin A

Whether Azadirachtin A, a potent insecticide of plant origin,[9] might also inhibit Pgp function was evaluated in the short-term assays of the restoration of the retention of DAU and R-123 in the MDR-P388 and MDR-CEM cells. With both MDR cell lines and at least up to 30 μg/ml, no increased retention of either fluorescent probes and no effect on the Par cell retention controls were found. Thus, Azadirachtin A did not detectably inhibit Pgp function.

Chemosensitization of MDR cells by ivermectin

Using the two pairs of MDR and Par tumor cell lines, ivermectin was compared with a few Pgp-directed modulators for its capacity to restore the cytostatic responses (cell growth inhibition) of the MDR cells, to the anti-cancer drugs which had been used for their selection to multidrug resistance. A range of modulator concentrations was assayed for the chemosensitizing effects (expressed as RDF calculated as described in Methods) and for direct cytostatic effects.

Restoration of the sensitivity of MDR-CEM cell growth to vinblastine. In culture medium (no modulator solvent), the MDR-CEM cells were highly resistant (more than 1000-fold) to vinblastine with a mean IC_{50} of 0.54 ± 0.08 μg/ml, in comparison with 0.51 ± 0.10 ng/ml for the Par-CEM cells. No complete reversion of that very strong MDR phenotype could be achieved with any modulator within a concentration range lacking direct cytostatic effects for the MDR or Par cells (Table 4). A roughly 200-fold sensitization was obtained with 3 μg/ml (about 2.4 μM) of the strongest Pgp modulators (SDZ PSC 833 and SDZ 280-446), while CsA or verapamil showed little activity. Both ivermectin preparations gave a 60-fold sensitization at the 3 μg/ml concentration (about 3.4 μM), thus being definitely less active than SDZ PSC 833, though more active than CsA, for the human MDR-CEM cells.

Restoration of the sensitivity of MDR-P388 cell growth to doxorubicin. In culture medium (no modulator solvent), the MDR-P388 cells showed a ≥ 300-fold doxorubicin resistance with a mean IC_{50}

Table 3. Restoration of the retention of DAU or R-123 in MDR-P388 cells by various modulators

Modulator (μg/ml)	Retention in MDR cells as percent of its retention in similarly treated Par cells[a]							
	0	0.03	0.1	0.3	1	3	10	30
DAU								
SDZ PSC 833	0 ± 0	5 ± 4	32 ± 1	70 ± 19	80 ± 13	104 ± 13	ND	ND
Ivomec™	0 ± 0	ND	ND	5 ± 7	19 ± 1	43 ± 1	60 ± 7	87 ± 36
IVM	0 ± 0	ND	ND	3 ± 4	8 ± 10	43 ± 1	52 ± 6	70 ± 1
R-123								
SDZ PSC 833	13 ± 2	8 ± 3	15 ± 1	37 ± 1	72 ± 10	96 ± 16	ND	ND
Ivomec™	15 ± 6	ND	ND	11 ± 2	12 ± 1	34 ± 2	67 ± 3	92 ± 16
IVM	10 ± 2	ND	ND	10 ± 2	14 ± 1	36 ± 1	71 ± 10	67 ± 17

[a]The results are shown as means (±SD) of two independent experiments, each assay being performed in duplicate. ND, not determined.

Table 4. Restoration of the vinblastine-sensitivity of MDR-CEM cells by various modulators

Cell line	Modulator (μg/ml)	RDF[a,b]			
		0.1	0.3	1	3
Par-CEM	SDZ PSC 833	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1*
	Ivomec™	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.1***
	IVM	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.0**
	SDZ 280-446	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1*
	CsA	ND	ND	1.0 ± 0.1*	1.1 ± 0.1**
	verapamil	ND	ND	1.3 ± 0.1	1.7 ± 0.1
MDR-CEM	SDZ PSC 833	1.2 ± 0.0	3.5 ± 0.6	76 ± 18	223 ± 90*
	Ivomec™	1.0 ± 0.0	1.6 ± 0.3	10 ± 4	61 ± 16**
	IVM	1.1 ± 0.1	1.4 ± 0.2	7 ± 1	60 ± 9*
	SDZ 280-446	2.9 ± 0.5	21 ± 4	88 ± 13	182 ± 48
	CsA	ND	ND	1.4 ± 0.1	17 ± 3
	verapamil	ND	ND	2.9 ± 0.5*	⩾ 17*

[a]Three to five independent determinations (in triplicate); ND, not determined. The vinblastine IC_{50}s for the Par-CEM cells were 0.59 ± 0.2 ng/ml in ethanol, 0.58 ± 0.1 ng/ml in the Ivomec™ solvent and 0.8 ± 0.2 ng/ml in DMSO, and the IC_{50}s for the MDR-CEM cells were 566 ± 70 ng/ml in ethanol, 571 ± 103 ng/ml in the Ivomec™ solvent and 540 ± 34 ng/ml in DMSO.
[b]Cell growth inhibition by the modulator alone was *negligible (< 20%), **medium or ***excessive (> 30%, limiting the significance of the RDFs).

of 6.0 μg/ml, in comparison with 18 ± 2 ng/ml for the Par-P388 cells. The significance of the comparisons of modulator was impaired by the high sensitivity of the P388 cell line pair to direct cytostatic effects of some modulators. Nevertheless, comparisons performed at the 1.0 μg/ml modulator concentration (which lacked severe cytostatic effects) showed that both ivermectin preparations gave some chemosensitization (5- to 8-fold), but that it was low in comparison with SDZ PSC 833 and SDZ 280-446 (Table 5). A higher modulatory activity of ivermectin was found at 3 μg/ml, but it was less obvious because of direct cytostatic effects. Thus, ivermectin was definitely less active than SDZ PSC 833 for the murine MDR-P388 cells. Comparisons of ivermectin with CsA was not possible as the cyclosporin already showed large direct cytostatic effects at 1 μg/ml.

Table 5. Restoration of the doxorubicin-sensitivity of MDR-P388 cells by various modulators

Cell line	Modulator (μg/ml)	RDF[a,b]			
		0.1	0.3	1	3
Par-P388	SDZ PSC 833	ND	0.9 ± 0.0	1.1 ± 0.1	ND
	Ivomec™	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.0	0.5 ± 0.1***
	IVM	0.9 ± 0.0	0.9 ± 0.1	1.0 ± 0.1	0.7 ± 0.2***
	SDZ 280-446	ND	0.9 ± 0.0	1.2 ± 0.2*	ND
	CsA	1.0 ± 0.1	1.1 ± 0.2**	1.0 ± 0.1***	0.6 ± 0.1***
	verapamil	ND	ND	1.0 ± 0.1	ND
MDR-P388	SDZ PSC 833	ND	97 ± 7	108 ± 10*	ND
	Ivomec™	1.2 ± 0.1	1.8 ± 0.3	8 ± 2	47 ± 8***
	IVM	1.2 ± 0.2	1.6 ± 0.3	5 ± 0.1	35 ± 5**
	SDZ 280-446	ND	66 ± 7	106 ± 5	ND
	CsA	1.9 ± 0.4	5 ± 3	82 ± 16**	236 ± 12***
	verapamil	ND	ND	4.0 ± 0.2	ND

[a]Two to four independent determinations (in triplicate): ND = not determined. The doxorubicin IC_{50} for Par-P388 cells was 17 ± 2 ng/ml in all three solvents (ethanol, the Ivomec™ solvent and the DMSO), and the IC_{50}s for MDR-P388 cells were 6540 ± 2208 ng/ml in ethanol, 6717 ± 1981 ng/ml in the Ivomec™ solvent and 7677 ± 2409 ng/ml in the DMSO.
[b]Cell growth inhibition by the modulator alone was *negligible (< 20%), **medium or ***excessive (> 30%, limiting the significance of the RDFs).

Discussion

Acute susceptibility to Ivomec™-mediated toxicity was found in animals with a specific BBB deficiency such as *mdr1a*^{KO} mice,[6] as well as in normal mice that had been pretreated with the potent Pgp-blockers SDZ PSC 833 or SDZ 280-446.[2] It was thus suggested that the widely used pesticide ivermectin might be a Pgp substrate, its access to the CNS being facilitated by an absence of functional Pgp molecules at the BBB level. It now appears that, like several other macrolides such as FK-506, ascomycin and rapamycin,[1] ivermectin is not just a substrate but that it is also a potent Pgp modulator. In contrast Azadirachtin A, another potent pesticide,[9] lacked any substantial capacity to inhibit Pgp function. This may have major consequences for the use of ivermectin as a pesticide on pets and for agriculture. The potential neurotoxicity of ivermectin obviously excludes its use for MDR reversion. Nevertheless, how potent a Pgp inhibitor ivermectin is should be known when man or animals with diminished Pgp function are at risk of being exposed to the pesticide.

In comparison with SDZ PSC 833, the capacity of ivermectin to inhibit Pgp function looked stronger in the short-term assays which measure Pgp probe retention (Figure 1) than in the long-term ones which measure the modulator-mediated sensitization of MDR cell growth to anti-cancer agents. Such differences of modulatory capacity in short-term and longer-term assays were observed with some polycyclic lactones and it may be due to the low stability of such macrolides in culture conditions.[1] This may also reduce the risk of ivermectin intoxication for man.

In comparison with other modulators, ivermectin modulatory capacity would rank somewhere between SDZ PSC 833 and CsA; a more precise ranking cannot be done because it depends on the different types of assays and cell lines. Differences of modulatory activities of ivermectin for the Pgp molecules expressed on human MDR-CEM and murine MDR-P388 cells might be related to the species differences. Considering MDR-CEM cells and only the purified ivermectin for comparisons with SDZ PSC 833, ivermectin was found less active for restoring the retention of Pgp probes [EC_{50} ratios: 2-fold (0.59/0.30) for DAU and 1.4-fold (6.7/4.8) for R-123 in MDR-CEM cells] and for increasing their sensitivity to vinblastine [comparable RDFs required 3 μg/ml ivermectin (60-fold) but only 1 μg/ml SDZ PSC 833 (76-fold)]. Though a weaker modulator than SDZ PSC 833, ivermectin thus shows substantial Pgp

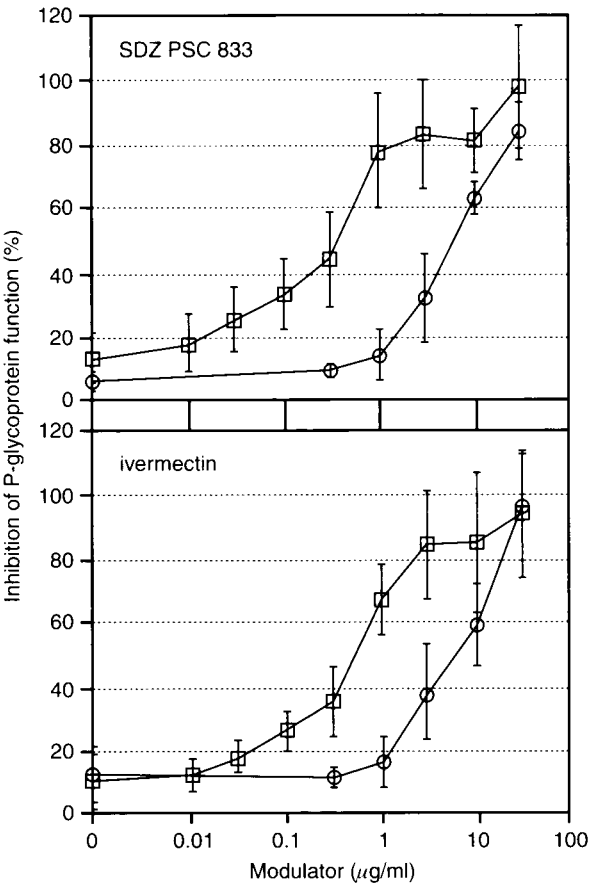

Figure 1. Dose dependence of Pgp function inhibition by ivermectin and by SDZ PSC 833. The cyclosporin and macrolide data are in the top and bottom diagrams, respectively. The modulator concentrations are shown on the *x*-axes as μg/ml (1 μg/ml = 1.14 μM for ivermectin and 0.82 μM for SDZ PSC 833). The inhibition of Pgp function in the MDR-CEM cells is shown on the *y*-axes as percentage (mean ± SD) of the Pgp probe retention (squares for DAU and circles for R-123) in similarly treated Par-CEM cells.

inhibitory properties, and one might consider to chemically alter the macrolide to reduce its neurotoxicity and enhance its Pgp modulator features.

Conclusion

The ivermectin toxicity found in SDZ PSC 833-treated mice[2] was interpreted as resulting from Pgp blockade by the cyclosporin then allowing free diffusion of the macrolide into the brain. This might lead to designing novel strategies of pharmacological intervention in the CNS, based on a Pgp-specific neutralization of the BBB function. Indeed, a variety of drugs designed for the treatment of CNS pathologies, from brain cancer to multiple sclerosis, are not

well taken up in the CNS and present physical features suggest they may be Pgp substrates. A major pharmacological interest of SDZ PSC 833 might thus be its capacity to allow the free diffusion of such drugs by causing the temporary neutralization of the Pgp-dependent component of the BBB. However, this strategy should now be modulated by the discovery that ivermectin was itself a potent inhibitor of Pgp function; thus, the ivermectin toxicity found in SDZ PSC 833-treated mice[2] might actually result from the *cumulative* inhibitory effects of ivermectin and SDZ PSC 833 on the Pgp molecules, rather than their inhibition by the latter only. Presumably, *saturation* of the effluxing capacity of the Pgp molecules expressed on the CNS capillary endothelial cells was as readily achieved by combining low doses of Ivomec™ and SDZ PSC 833 as by a higher dosage of Ivomec™ alone.

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