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DECREASED BIOTOLERABILITY FOR IVERMECTIN AND CYCLOSPORIN A IN MICE EXPOSED TO POTENT P-GLYCOPROTEIN INHIBITORS

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SDZ PSC 833 or SDZ 280-446 are strong blockers of the function of class I *mdr* gene-encoded P-glycoprotein molecules, which were developed for the reversal of multi-drug-resistance of tumor cells. When treated with such drugs, normal mice may display hypersensitivity to cyclosporin A and ivermectin. The recorded signs of acute toxicity are compatible with alterations of the murine central nervous system functions and with earlier data suggesting that P-glycoprotein expressed at the murine blood–brain barrier might be involved in the exclusion of cyclosporin A or ivermectin from brain tissue.

The class I *mdr* gene-encoded P-glycoprotein (Pgp) molecules are members of a family of the ATP-binding cassette (ABC) transporter proteins. These transmembranous proteins transport a variety of compounds, from ions to small hydrophobic drugs and peptides. Unlike other ABC family members, Pgp molecules possess a very broad substrate specificity, the transported compounds, among which are anti-cancer drugs, showing neither structural nor functional relationships besides their high lipophilicity (Gros and Buschman, 1993). When Pgp molecules are over-expressed by various tumor cells, they efficiently efflux anti-cancer drugs which are diffusing through the tumor cell plasma membrane; this leads to the emergence of a multi-drug resistance (MDR) phenotype, which is often responsible for failure of chemotherapy (Moscow *et al.*, 1994). The most efficient way to achieve pharmacological reversal of tumor cell MDR is to perform the cytostatic-based chemotherapy in combination with the little or non-immunosuppressive cyclosporin SDZ PSC 833 or cyclopeptolide SDZ 280-446 (Loor, 1994). Such resistance-modulating agents (RMA) cause a persistent inhibition of the class I *mdr*-encoded Pgp molecules of tumor cells (Boesch and Loor, 1994). Cyclosporin A (CsA) inhibits the pumping function of Pgp molecules in a competitive and reversible way, presumably because it is a slow Pgp substrate (Saeki *et al.*, 1993).

Tolerability studies with normal mice exposed to combinations of doxorubicin and SDZ PSC 833 showed that the RMA could increase murine sensitivity to doxorubicin. Nevertheless, death resulting from a direct or RMA-mediated over-dosage of doxorubicin was not caused by the lymphotoxicity or the myelotoxicity of the chemotherapy. Particularly, adoptive transfer studies showed that heavy (death-causing) chemotherapy treatments of donor mice left enough stem cell potential for the rehabilitation of irradiated recipients. In contrast, large bone marrow cell grafts from normal mice could not rescue from death mice which had been over-exposed to doxorubicin given in combination with SDZ PSC 833 (Froidevaux and Loor, 1994).

Such toxicities might come from any one of various normal cells and tissues which express class I *mdr*-encoded Pgp (O'Brien and Cordon-Cardo, 1991). Among these are the apical surfaces of the secretory epithelium of the jejunum and colon, the proximal tubular epithelium of the kidney, the bile canaliculi of the liver and pancreatic small ductule epithelium and the blood surface of capillary endothelial cells in brain and testis. Such tissue locations and polarized cell expression support the concept that Pgp plays a role in protecting the body from toxic agents at various levels, particularly a higher clearance by transport into bile, urine and feces. Inhibition of the transport of some Pgp substrates could be obtained with SDZ PSC 833 using representative cell lines or animal models (Böhme *et al.*, 1993; Speeg and Maldonado, 1994). A function of Pgp expression can be expected to reduce the absorption of toxic agents from the gut, as well as to block their access to critical organs such as brain and testis, which must be drug-free sanctuaries.

The function of Pgp expression at the blood–brain barrier (BBB) in mice (Shirai *et al.*, 1994; Tatsuta *et al.*, 1992) was further stressed by other pilot tolerability studies in our laboratory: normal mice showed some signs of neurotoxicity when CsA (at dosages known to give low to good immunosuppression) was given orally together with SDZ 280-446 (at dosages known to be efficient in combined chemotherapy of MDR-tumor). Recently, *mdr1a* knock-out (*mdr*1a^{KO}) mice, which are lacking one of the two murine class I *mdr*-encoded Pgp isotypes, were described as having a BBB deficiency, particularly evidenced by a much increased sensitivity to the ivermectin (IVM) pesticide (Schinkel *et al.*, 1994). Since both IVM (Fisher and Mrozik, 1992) and CsA (Cefalu and Pardridge, 1985; Liu *et al.*, 1994) may be neurotoxic, we performed further tolerability studies in mice of oral (per os [p.o.]) SDZ 280-446 or SDZ PSC 833 in combination with p.o. CsA or with intravenous (i.v.) or intraperitoneal (i.p.) IVM.

MATERIAL AND METHODS

Drug sources and dosages

CsA, SDZ PSC 833 and SDZ 280-446 were kindly provided by Sandoz (Basel, Switzerland); they were dissolved alone or in combination in the "placebo G" vehicle (a solution of Labrafil/ethanol/corn oil, 4:2:4, w/w; Sandoz). IVM was bought as Ivomec (Merck Sharpe and Dohme, Haarlem, The Netherlands), an injectable solution containing 1% IVM (10 mg/ml). For control assays, the IVM solvent was prepared with glycerol formal (40%) and propylene glycol (60%) from Sigma (St. Louis, MO). For experimentation, all drug and control solutions were diluted with PBS just before use to concentrations allowing dosing at a ratio of 10 µl/g of mouse. IVM, CsA, SDZ PSC 833 and SDZ 280-446 dosages and drug combination protocols were chosen on the following bases.

For IVM tolerability studies, the choice of the 1 mg/kg dosage mostly used for the combined drug treatment was based on data reported for IVM toxicity in normal mice (LD_{50} of 30 mg/kg for i.p. IVM [Fisher and Mrozik, 1992] and 50–60 mg/kg for p.o. IVM [Schinkel *et al.*, 1994]) and in *mdr*1a^{KO} mice (an LD_{50} of 0.7–0.8 mg/kg for p.o. IVM [Schinkel *et al.*, 1994]).

For CsA tolerability studies, we used daily p.o. dosages of 5–100 mg/kg CsA for 5 consecutive days. This was based on data showing that, in mice, high dosages of p.o. CsA, from 20 mg/kg in OF_1 mice to 100 mg/kg in C57BL/6 mice, were required to bring substantial immunosuppression (Hiestand and Gubler, 1988).

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For the concentrations of CsA, SDZ PSC 833 and SDZ 280-446 used as RMA, we used the single 50 mg/kg RMA dosage on a daily p.o. administration basis for up to 5 consecutive days. Indeed, our earlier studies of MDR-tumor chemotherapy, combining anti-cancer drugs (i.p.) and RMA (p.o.) for 5 consecutive days, showed that 25–50 mg/kg RMA were found to be the most suitable p.o. dosages (Boesch *et al.*, 1991; Loor *et al.*, 1992).

Mouse treatment protocol

C57BL/6 (B6) mice were bred in our animal colony, while their (C57BL/6 × DBA/2) F_1 hybrids ($B6D2F_1$) were bought from Iffa-Credo (l'Arbresle, France) and housed in our isolators with sterile bedding, food and water for a few days before the assay.

In the case of IVM tolerability studies, mice received a single i.p. or i.v. injection of IVM or its control solvent at day 0, 4 hr after the first of up to 5 p.o. administrations of RMA or its control vehicle at 1-day intervals. Most mice were treated with an IVM dosage of 1 mg/kg (1 μg/10 μl/g); thus, mice weighing 20 g required 20 μg, which were injected i.p. or i.v. in the tail vein as 200 μl of a 100 μg/ml IVM solution. RMA-treated mice received the RMA p.o. at 50 μg/10 μl/g.

In the CsA tolerability studies, mice received various CsA dosages and/or 50 mg/kg RMA (SDZ 280-446 or SDZ PSC 833), dissolved together in the placebo G vehicle. Drug mixtures were given p.o. to $B6D2F_1$ mice for 5 consecutive days, all mice receiving the same volume of the placebo G vehicle.

Survival and weight were recorded daily. The first treatment being done on the morning of day 0, survival was counted as half a day if mice died during the same day and as 1 full day if mice were still alive on the next morning, and so on for further days. Other behavioral peculiarities were also recorded.

RESULTS

Several independent experiments, each with very few mice, compared the biotolerability of CsA or IVM taken as Pgp substrates alone or in combination with strong RMA. Each individual mouse studied gave a "case report" describing the treatment sequels. Drug regimen protocols of interest were repeated and data were cumulated as mouse groups. In the case of CsA, all assays were performed with 6 to 8 week-old $B6D2F_1$ females, and the results were pooled as one mouse group per drug combination (Table I) since all assays shared the same protocol consisting of simultaneous p.o. administration for 5 consecutive days of a vehicle containing CsA and/or RMA (or no drug for controls). In the case of IVM, we explored some individual parameters of mouse variability (sex, age, strain) and of administration protocol (i.p. or i.v., dosage, timing). Results were pooled as one mouse group per drug administration protocol (Table II), other parameters either showing no substantial influence on the results or being described in the text.

Toxicity of CsA in combination with SDZ 280-446 or SDZ PSC 833

CsA was thus taken as slow Pgp substrate rather than as RMA, and its biotolerability was compared when given alone or together with much stronger RMA (Table I).

SDZ 280-446 and SDZ PSC 833 at 50 mg/kg were well tolerated by the $B6D2F_1$ mice (groups 2 and 3), with only 1 delayed mouse death in each group and no earlier sequel recorded. CsA was well tolerated up to 50 mg/kg when used alone (groups 4, 6, 8 and 11), with only 2 early mouse deaths after wasting or paralysis, but 4 of 5 mice treated with 100 mg/kg CsA (group 14) showed early paralysis and death. In combination with SDZ 280-446 (at 50 mg/kg), CsA was well tolerated up to 10 mg/kg. However, when combined at 20 or 50 mg/kg with SDZ 280-446 (groups 9 and 12) or SDZ PSC 833 (groups 10 and 13), CsA treatment displayed substantial toxicity for at least half of the mice: recorded toxic sequels were mild and reversible for some mice as they recovered without apparent development of delayed sequels but severe for other mice as they died most often during the treatment or soon after it. The most common sign of mild toxicity was

TABLE I – TOXICITY OF CsA IN COMBINATION WITH SDZ 280-446 OR SDZ PSC 833[1]

Mouse group	p.o. Drugs (mg/kg/day)		Mouse numbers		
			Sick/ treated	Sequels	
	CsA	RMA		Rev.	Irr.
1	0	V	0/36	0	0
2	0	280	1/24	0	1*
3	0	PSC	1/21	0	1**
4	5	V	0/9	0	0
5	5	280	0/10	0	0
6	10	V	1/10	0	1
7	10	280	0/10	0	0
8	20	V	1/35	0	1
9	20	280	16/24	5	11***
10	20	PSC	11/21	6	5
11	50	V	0/15	0	0
12	50	280	3/6	0	3
13	50	PSC	10/10	5	5
14	100	V	4/5	0	4
15	100	PSC	5/5	1	4

[1]B6D2F1 mice (6 to 8 week-old females) were treated p.o. with a mixture of CsA (range of doses) and RMA (50 mg/kg, either SDZ 280–446 [280] or SDZ PSC 833 [PSC]; V = vehicle only). Numbers of mice showing sequels are shown as ratio of total numbers of treated mice. Sequels were subdivided as irreversible (Irr.), leading to death or requiring euthanasia, or as spontaneously reversible (Rev.), mostly occurring at day 3 of treatment, with no further sequel for the next 2 months. Reversible sequels were wasting (hyperventilation, pilo-erection, drowsiness, prostration). Irreversible sequels were death after wasting, tremor, convulsions and paralysis. Death occurred mostly between days 3 and 7, except for 3 mice which died at days *38, **14 and ***49.

TABLE II – INCREASED IVM TOXICITY IN RMA-TREATED MICE[1]

Mouse group	IVM (mg/kg)	RMA (50 mg/kg p.o.)	Dead/total after	
			IVM i.v.	IVM i.p.
1	0	V	0/23	0/7
2	0	PSC	0/13	0/3
3	0	280	0/8	0/3
4	0	CsA	0/6	
5	0.2	V	0/5	0/3
6	0.2	PSC	0/6	
7	0.2	280		0/3
8	0.5	PSC	0/2	
9	0.6	V	0/5	
10	0.6	PSC	1/5	
11	0.8	PSC	3/3	
12	1	V	0/21	0/11
13	1	PSC	15/15	1/5
14	1	280	8/8	3/6
15	1	CsA	0/4	
16	3	V	0/7	0/1
17	3	CsA	0/2	
18	10	V	4/10	1/4
19	10	PSC		2/2
20	≥ 15	V	5/5	10/10

[1]Mice were exposed to a single injection of IVM (various dosages; 0 = solvent only) 4 hr after the first p.o. administration of the RMA (50 mg/kg, either SDZ 280–446 [280], SDZ PSC 833 [PSC] or CsA; V = vehicle only; see text for details). Numbers of mice showing early death (after coma) or prolonged coma (followed by euthanasia), most often occurring within hours of IVM injection, are shown as ratio of total numbers of treated mice.

wasting with hyperventilation, pilo-erection, drowsiness and prostration. Wasting was also observed in cases of acute toxicity, together with other signs preceding death: convulsions, tremor and partial or complete paralysis of the legs.

Toxicity of i.v. or i.p. IVM in combination with SDZ 280-446 or SDZ PSC 833

All data were recorded independently as case reports. No differences were found between B6 mice and their (B6 × D2)F_1 hybrids (B6D2F1), males and females or young adults (6 to 8 week-old) and older adults (up to 16 week-old). As the results depended on the administration protocol (i.p. or i.v. IVM, dosage, timing), results were pooled as one mouse group per protocol (Table II). In the experiments of Table II, IVM was injected into mice 4 hr after p.o. gavage with RMA (or its vehicle in controls) as pharmacokinetics studies had shown that SDZ PSC 833 bioavailability in serum would peak about 2 hr and stabilize at a high plateau level 3 to 4 hr after gavage.

IVM alone (groups 5, 9, 12, 16, 18 and 20) was well tolerated since no mice showed sequels after a single i.v. injection up to 3 mg/kg or a single i.p. injection up to 10 mg/kg. Death occurred in all mice injected i.v. or i.p. with ≥ 15 mg/kg IVM. With the 10 mg/kg IVM dosage, death occurred in 1 of 4 i.p.-injected mice and 4 of 10 i.v.-injected mice. The data suggest that the LD_{50} for a single IVM injection was higher than 10 mg/kg. Before death, mice showed signs of acute toxicity from loss of balance to early coma (within minutes of IVM injection). This CNS-type toxicity was IVM-related as none of the mice injected with the IVM solvent only (propylene glycol and glycerol formal) showed significant sequels; only mice exposed to the 2 highest i.v. tested dosages (20 and 30 mg/kg) briefly responded by some generalized muscular contractions within 1 min after injection but did not show any detectable sequels when later followed for several days (those IVM [Ivomec] solvent controls were performed with similar numbers of mice as the IVM-treated ones, but they are not shown in Table II).

When IVM was given 4 hr after the first of 2 p.o. RMA gavages (50 mg/kg, given at 24-hr intervals), an IVM dose-dependent acute toxicity appeared in some mice, with tremor and coma preceding early death within 12 hr (most cases), 1.5 days (few cases) or 3.5 days (single case) after IVM injection. Up to 0.5 mg/kg IVM (groups 6, 7 and 8), no death or other sign of severe toxicity was recorded when mice were followed for about 2 months after treatment. Death began with 0.6–0.8 mg/kg i.v. IVM combined with SDZ PSC 833 (groups 10 and 11) and 1 mg/kg i.p. IVM combined with SDZ PSC 833 or SDZ 280-446 (groups 13 and 14). While 1 mg/kg i.v. IVM killed all mice pre-treated with SDZ PSC 833 or SDZ 280-446 (groups 13 and 14), none pre-treated with CsA died (group 15), though the p.o. administration of CsA was prolonged for 5 consecutive days.

Because Pgp expression is developmentally regulated, we included in the IVM tolerability study a few very young mice which were not weaned. The 18 to 35 day-old mice did not appear to be more sensitive to IVM alone than adult mice. Furthermore, the largest amounts of IVM solvent alone (needed to inject a 30 mg/kg IVM dosage) could be injected i.p. or i.v. without any detectable sequels. Due to the small size of such mice, the combination of IVM with p.o. RMA could not be tested. We did not find differences for IVM ± RMA tolerability related to either sex or genetic context (B6 and B6D2F_1 are hemigenic).

Finally, the emergence and persistence of IVM hypersensitivity after RMA gavage was explored. B6D2F_1 females (7 to 8 week-old) received a single p.o. administration of SDZ PSC 833 (50 mg/kg) and a single i.v. IVM (1 mg/kg) injection 1, 2, 4, 8, 10, 14, 24, 30 and 48 hr later (3 mice per group). As early as 1 hr and up to 10 hr after SDZ PSC 833 gavage, all mice in each group showed IVM hypersensitivity and early death, but not 14 hr after RMA gavage.

DISCUSSION

We shall restrict the use of the term "RMA" for the strongest compounds (SDZ PSC 833 and SDZ 280-446) which can virtually "knock out" Pgp function, thus excluding CsA, which will be termed as such. Both transcellular transport models using epithelioma monolayers with polarized Pgp expression (Augustijns *et al.*, 1993; Saeki *et al.*, 1993) and intracellular retention assays using MDR-leukemia cells (Didier *et al.*, 1995) showed that CsA can be transported by the Pgp pump; yet, by being a slow substrate of the Pgp pump, it can also behave as a competitive and easily reversible inhibitor of Pgp function. So far, the evidence for IVM being also a Pgp substrate is indirect only (Schinkel *et al.*, 1994), but data from our laboratory suggest it may behave like CsA (data not shown).

In the course of tolerability studies for RMA and CsA or IVM combinations in the mouse, we thus observed various sequels, suggestive of CNS toxicity. Particularly, acute death was found for IVM when combined with RMA treatment. Since both CsA and IVM showed dose-dependent toxicity and RMA treatment can increase the plasma bioavailability of various anti-cancer drugs, it could be suggested that a few-fold increased CsA or IVM bioavailability might be leading to CNS dysfunction. Alterations of anti-cancer drug pharmacokinetics resulting in increased plasma bioavailability are known to occur after SDZ PSC 833 administration, but they were not stronger than those seen with CsA. The known cause for bioavailability increases, an interference at the level of the P450-based detoxification system, suggests that CsA might increase in that way its own plasma bioavailability; whether the latter was higher when used in combination with SDZ PSC 833 than when used alone was not tested. This simple explanation may not be the sole cause since, when CsA or IVM were used alone, much higher dosages were required to show significant toxicities than when used in combination with RMA. Furthermore, in spite of its pharmacokinetic effects, CsA could not hypersensitize the mice to IVM. It thus appears that use of the strong RMA was required to prime the mice to hypersensitivity to CsA or IVM.

The neurotoxicity might thus be due to excessive diffusion of the RMA into the brain. Thus, CsA or IVM might enhance plasma RMA bioavailability and/or keep Pgp molecules so much engaged in their own efflux that not enough Pgp function would be available for RMA binding, thus avoiding leakage through the BBB from blood to brain. However, no detectable signs of CNS malfunction were observed for RMA alone in mice up to at least 2-fold higher dosages than those tested here (Boesch *et al.*, 1991; Loor *et al.*, 1992).

Since the neurotoxicity of CsA and IVM is low in mammals with an intact BBB (Cefalu and Pardridge, 1985; Fisher and Mrozik, 1992; Schinkel *et al.*, 1994), it is most likely that RMA helped CsA or IVM to cross the BBB rather than the contrary, this leading to the different symptoms we recorded. The simplest hypothesis is that blockade of the BBB-associated Pgp by a strong RMA would allow unrestricted penetration of CsA and IVM into the brain tissue. Therefore, when CsA or IVM was used in combination with a strong RMA, any observed CNS sequels should be essentially mediated by the companion drug, CsA or IVM, whose BBB by-pass would be facilitated by the RMA.

By favoring CsA penetration in the CNS, the RMA would lead to neurotoxicity: CsA uptake by nerve cells in the brain might eventually cause, through calcineurin deviation by CsA–cyclophilin complexes, alterations of the normal cycling of Dynamin I GTPase activities in the nerve terminals (Liu *et al.*,

1994). The mechanisms of CsA-mediated neurotoxicity are, however, unclear, which impairs further interpretation of the symptoms shown by the mice.

At variance, the IVM effects on nerve transmission have been more extensively studied (Fisher and Mrozik, 1992). IVM binding to the GABA A receptors (GABA-R) in the CNS has agonistic effects as it enhances GABA binding to its receptor. The resulting conformational change of GABA-R induces Cl^- channel opening, with a consequent hyperpolarization leading to nerve signalling inhibition and the observed toxicity. Because GABA-R are exclusively found in the CNS and IVM does not cross the BBB, it is normally well tolerated in most mammals, except those with an incomplete, developing BBB such as neonatal rats (Fisher and Mrozik, 1992) and those with specific BBB deficiency such as *mdr*1a^{KO} mice (Schinkel *et al.*, 1994). Studies on the renal expression of class 1 *mdr* genes during post-natal murine development showed that the Pgp molecules and their mRNA, though undetectable at birth, were expressed abruptly at the adult levels between 2 and 3 weeks of age (Dutt *et al.*, 1992). No such information was available for the BBB, but most of our studies being performed with young adult mice and no direct or RMA-mediated IVM hypersensitivity being found in our youngest studied mice (3 week-old), we assume that the BBB displayed normal levels of Pgp function. Therefore, our studies suggest that 50 mg/kg of strong Pgp-directed RMA such as SDZ 280-446 and SDZ PSC 833, but not CsA, can break the Pgp-dependent component of the murine BBB: in their presence, an IVM dosage which was normally well tolerated by the mice (a single i.v. 1 mg/kg IVM injection) caused acute CNS dysfunction (convulsion, paralysis, coma) within a few hours of injection. Thus, when SDZ PSC 833 or SDZ 280-446 inhibited the function of the BBB-associated Pgp, IVM could reach the GABA-R in the CNS. Efficient BBB opening by SDZ PSC 833 lasted for at least 10 hr after its p.o. administration since an i.v. IVM injection made at that time still showed full toxicity.

The reappearance of IVM resistance, within less than 1 day after RMA treatment, may be due either to a dissociation of RMA from the Pgp, to new Pgp expression at a time the RMA bioavailability had already dropped so much that it could not block Pgp efficiently enough or to the emergence of a non-Pgp-mediated mechanism of IVM resistance. Definitely, the former 2 alternatives do not concern the *mdr*1a^{KO} mice, whose deficiency is genetic, but the latter alternative should take place in *mdr*1a^{KO} mice and protect them, unless a blockade of *mdr*1a-encoded molecules would be a stress needed for induction of that other (speculative) mechanism of IVM resistance.

At variance with stronger RMA such as SDZ PSC 833 or SDZ 280-446, CsA was unable to neutralize the BBB Pgp function to an extent such that IVM would be symptomatic, suggesting that CsA (at least up to 50 mg/kg) left enough Pgp function intact to exclude IVM access to the brain tissue. Possibly, higher dosages of CsA than those used here might have led to substantial Pgp neutralization and IVM toxicity. However, the interpretation of such experiments would be impaired by the complication that neurotoxicity might then arise from CsA itself, as found when using CsA alone at 100 mg/kg.

Although both our experiments and the study of *mdr*1a^{KO} mice (Schinkel *et al.*, 1994) point in the same direction—*i.e.*, an involvement of Pgp as an essential BBB component—detailed comparisons of the results are impaired by the different IVM administration procedures, as well as by the different mouse strains used, which do not necessarily show similar drug pharmacokinetics and BBB quality. Moreover, another potential difference might be the involvement of *mdr*1b-encoded Pgp molecules in IVM efflux, whereas RMA such as SDZ PSC 833 and SDZ 280-446 probably interfered with the functions of both *mdr*1a- and *mdr*1b-encoded Pgp molecules.

A Pgp neutralization at the BBB level has potential implications for the cancer patient. Our data show a large difference between CsA on the one side and SDZ PSC 833 or SDZ 280-446 on the other side in their capacities to inhibit the function of Pgp at the BBB level. Up to very high dosages at which it started to display toxicity, CsA could not achieve any detectable BBB Pgp inhibition, at least by using the IVM acute toxicity as a read-out. Therefore, it would appear that, *in vivo*, only the most effective Pgp blockers are able to substantially inhibit Pgp function. If these results concerning BBB Pgp are to be extrapolated to tumor cell Pgp, this means that current attempts using CsA to restore anti-cancer drug sensitivity of MDR-tumors in patients will essentially fail or that, if they work, it might be essentially through the pharmacokinetic interactions which increase anti-cancer drug bioavailability (and toxicity). In the case of SDZ PSC 833 (or SDZ 280-446), pharmacokinetic interactions may also play a role, but additional effects definitely come from Pgp neutralization, a conclusion we had already drawn from earlier studies on tumor-bearing mice. Although *in vitro* it was possible to obtain virtually the same MDR reversion level, at least for tumor cell lines with moderate Pgp expression, with CsA or its stronger congeners, by simply using much higher concentrations of CsA than SDZ PSC 833 or SDZ 280-446 (Gavériaux *et al.*, 1991; Loor *et al.*, 1992), this cannot be considered *in vivo*, particularly in clinical practice. Indeed, increasing the CsA concentrations enough to neutralize Pgp function as efficiently as with SDZ PSC 833 or SDZ 280-446 would require such large amounts of CsA that acute toxicities would follow, particularly neurotoxicity, hepatotoxicity and nephrotoxicity.

In conclusion, SDZ PSC 833 and SDZ 280-446 may open a novel window for BBB crossing by drugs and new ways for pharmacological interventions in the brain: an RMA treatment might be used for the temporary and reversible BBB opening to selective drugs. For instance, RMA-mediated Pgp blockade might be used to increase the brain penetration of a range of cytostatic Pgp substrate drugs for better brain tumor treatment and possibly even to confer access into the brain of novel anti-cancer drugs which might be particularly designed for some types of brain tumors.

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