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# Pharmacological Considerations in the Modulation of Multidrug Resistance

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## INTRODUCTION

MULTIDRUG RESISTANCE (MDR) is the phenomenon in which cancer cells become resistant to an array of chemotherapeutic agents with dissimilar structures and mechanisms of action [1]. MDR can be mediated by the overexpression of a multidrug transporter, the best studied of which is termed P-glycoprotein (Pgp) [2–6]. Pgp is a 170 kd plasma membrane protein with twelve transmembrane domains and two intracellular ATPase sites [7]. Pgp has broad specificity as an energy-dependent efflux pump and effectively transports a variety of amphipathic compounds out of the cell. Substrates for Pgp include many anticancer drugs, most notably those derived from natural products (Table 1). Many intrinsically chemoresistant cancers overexpress Pgp *de novo*. Other classically chemosensitive malignancies such as breast, ovarian and haematolymphoid cancers seldom express Pgp at diagnosis, yet have a high proportion of cases overexpressing Pgp upon relapse. Several studies have found Pgp expression to be predictive of poor response to chemotherapy and decreased overall survival (see reviews [8–10]).

Expression of Pgp also occurs in certain normal tissues (Table 2). Its presence on the apical surface of cells lining the lumen of large and small bowel, bile canaliculi and proximal renal tubules suggests that it may play a role in the excretion of potentially toxic xenobiotics [11]. Furthermore, the Pgp expressed along the intraluminal surface of vascular endothelium comprising the blood–brain barrier, placenta and blood–testes barrier may play a role in protecting the CNS (central nervous system), fetus and germinal cells from toxic substances in the circulation [12, 13]. Its function in other normal tissues remains a matter of conjecture.

The calcium channel blocker, verapamil, was the first non-cytotoxic agent found to inhibit the Pgp efflux pump *in vitro* [14]. Soon thereafter, numerous other currently approved medications were shown to be modulators of drug resistance *in vitro* (see Table 3). The potential clinical benefit to be derived from a potent Pgp inhibitor has spawned a new field of drug development and clinical research on the modulation of multidrug resistance (MDR). The purpose of this paper is to review the clinical pharmacology of selected Pgp inhibitors and to assess the impact of normal tissue Pgp inhibition on the disposition and elimination of chemotherapeutic agents.

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*Table 1. Chemotherapeutic agents which are substrates for the P-glycoprotein efflux pump*

| *Vinca alkaloids* | *Anthracyclines* | *Other* |
|---|---|---|
| Vinblastine | Doxorubicin | Mitoxantrone |
| Vincristine | Daunorubicin | Actinomycin-D |
| Vinorelbine | Epirubicin | Amsacrine |
| | Idarubicin | Trimetrexate |
| | | Topotecan* |
| | | Mithramycin* |
| | | Mitomycin C* |
| *Taxanes* | *Epipodophyllotoxins* | |
| Paclitaxel | Etoposide | |
| Docetaxel | Teniposide | |

* Low level of crossresistance to these agents.

*Table 2. Normal tissues which express P-glycoprotein*

Liver: lumenal surface of bile canaliculi
Kidney: lumenal surface of proximal tubules
Colon and small intestine: mucosa lining lumen
Adrenal cortex
Pancreas: ducts
Capillary endothelium of:
  CNS (blood–brain barrier)
  Testis (blood–testicular barrier)
  Placenta
Reactive mesothelial cells of malignant pleural and peritoneal effusions
Haematopoietic cells
  Macrophages
  Activated T-cells
  Pluripotent "stem cells" (CD34+)

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## CELLULAR PHARMACOLOGY OF MDR MODULATORS

Just as the anticancer substrates for Pgp transport are diverse in chemical structure and function, so too are the many Pgp inhibitors (see Table 3). Molecular characterisation of the drug binding sites within Pgp remains elusive, although analysis of *MDR1* mutations implicates several hydrophobic residues of the transmembrane regions of the protein. Photo-

*Table 3. MDR modulators and levels required to reverse MDR* in vitro

| "First generation" MDR modulators | |
|---|---|
| *Calcium channel blockers* | *Cyclosporins* |
| Verapamil (6–10 μM) | Cyclosporine A (2–4 μM) |
| Nifedipine (3 μM) | *Phenothiazines* |
| Bepridil (4 μM) | Trifluoroperazine (3–15 μM) |
| Nicardipine (3 μM) | Fluphenazine (3–4 μM) |
| *Other cardiovascular drugs* | *Hormones* |
| Amiodarone (5–10 μM) | Tamoxifen (~ 10 μM) |
| Dipyridamole (8–10 μM) | *N*-desmethyltamoxifen (~ 10 μM)* |
| Quinidine (4–7 μM) | 4-OH-tamoxifen (~ 10 μM)* |
| *Anti-malarials* | Toremifine (10 μM) |
| Quinine (4–6 μM) | Progesterone (8 μM) |
| Quinocrine (5 μM) | *Other* |
| Cinchonine (5–10 μM) | Terfenadine (3–5 μM) |
| *Antibiotics* | Cremaphor-EL (0.1%) |
| Cefoperazone (1000 μM) | Benzquinamide (20–40 μM) |
| Ceftriaxone (1000 μM) | Estramustine (10–20 μM) |
| Erythromycin (650 μM) | FK 506 (6–12 μM) |

| "Second generation" MDR modulators | |
|---|---|
| PSC 833 (0.5–2 μM) | *Other experimental agents* |
| Dexverapamil (6–10 μM) | Liposomal encapsulation |
| Dexniguldipine (1–2 μM) | – doxorubicin, paclitaxel |
| S9788 (2 μM) | Monoclonal antibodies |
| Rapamycin (2.5–5 μM) | – MRK-16 (10 μg/ml) |
| GF120918 (0.05–0.1 μM) | Antisense *mdr* 1 oligonucleotides |
| SR33557 (30 μM) | |

* Metabolites of tamoxifen. Reproduced from Lum BL, *et al.*, *Cancer* 1993, Vol. 72, pp. 3502–3514. Copyright © 1993 John Wiley and Sons Inc. Reprinted by permission of Wiley-Liss, a division of John Wiley and Sons, Inc.

affinity labelling studies have been used to demonstrate direct interaction between MDR reversal agents and Pgp [15]. Most of these modulators (e.g. verapamil, cyclosporin A (CsA), quinidine and tamoxifen) are themselves substrates for the Pgp efflux pump. Notable exceptions include the new cyclosporin analogue PSC 833, and anti-Pgp monoclonal antibodies such as MRK-16. Intracellular accumulation of PSC 833 is not altered by expression of Pgp, nor is it affected by the concurrent administration of other Pgp inhibitors or substrates [Dr D. Cohen, Sandoz Research Institute, East Hanover, New Jersey, U.S.A.]. Preclinical studies showing that MRK-16 and CsA are synergistic in their inhibition of Pgp suggest that separate but functionally complementary sites could be targeted to inhibit the efflux pump [16].

It has become apparent that the primary pharmacological function of the "off the shelf" drugs which are the first generation of Pgp inhibitors has little to do with their ability to reverse drug resistance. For example, the MDR modulation potency for verapamil and its analogues does not correlate with their potency as calcium channel blockers [17]. Tamoxifen modulates Pgp-expressing cells *in vitro* similarly in cells lacking oestrogen receptors as in cells bearing these receptors [18]. CsA is a potent immunosuppressive due to its binding to the cytoplasmic proteins termed cyclophilins. The cyclosporin analogue, PSC 833, neither binds to cyclophilins nor has any detectable immunosuppressive properties, yet PSC 833 is 2–10-fold more potent a modulator than CsA *in vitro*.

Most studies of the cellular pharmacology of MDR modulators are performed in tissue culture with media containing 5–15% fetal calf serum. However, serum proteins can have significant effects on some Pgp inhibitors and higher concentrations of serum can effectively render some inactive [19, 20]. Thus, it is desirable that serum protein interactions be studied in preclinical studies and that such effects be taken into account in the design of phase I studies, particularly when setting target serum levels of modulating drugs. Ideally, phase I studies should include *ex vivo* bioassays of MDR modulation [21]. Serum from patients treated with modulators can be assayed for an ability to modulate MDR *in vitro* either in traditional cytotoxicity assays or drug accumulation studies using MDR cell lines.

It is also important to note that currently available Pgp inhibitors appear to have no significant effect on resistance mediated by other newly described efflux pumps (e.g. MRP [22]). The extent to which non-Pgp-mediated MDR contributes to clinical drug resistance remains to be determined [23].

## CLINICAL PHARMACOLOGY OF MODULATORS ALONE

Early clinical modulation trials necessarily used first generation modulators, drugs approved for other indications, which had the advantage of easy availability as well as known toxicity profiles. However, it soon became clear that much higher than standard doses of these agents were required to achieve serum levels sufficient to inhibit Pgp. For verapamil, complete reversal of Pgp-mediated resistance occurs *in vitro* at concentrations of 8–10 μM. Unfortunately, using continuous infusion verapamil, the highest steady state plasma levels that could be achieved were < 5 μM. Not surprisingly, dose limiting toxicity was cardiovascular with heart block, congestive heart failure and hypotension requiring intensive care monitoring at those doses [24]. Interestingly, verapamil's own metabolism offers it a potential advantage in MDR modulation. Verapamil is extensively metabolised in the liver by P450 enzymes (CYP3A4 and CYP1A2) to two major metabolites, D-617 and norverapamil [25]. Norverapamil retains potency as a Pgp inhibitor and if serum levels of verapamil and norverapamil are summed, concentrations in the range of 6–8 μM can be achieved. These levels are just at the lower threshold of the desired concentrations. However, verapamil consists of a racemic mixture of dextrorotatory and levorotatory isomers. The dexverapamil enantiomer has significantly less effect on cardiac conduction than does the levo enantiomer [26], yet is equipotent in its ability to inhibit the Pgp efflux pump [27]. Dexverapamil has thus been proposed as an equipotent, less cardiotoxic modulator which might achieve the desired plasma concentrations with acceptable toxicity. Thus far, hypotension remains dose limiting and plasma levels that have been obtained at maximum tolerated doses have yielded only a modest increase of 1–2 μM above those attained with racemic verapamil. Specifically, in studies by Wilson and associates [28] and Motzer and associates [29], mean trough levels of dexverapamil were 1.1 μM and 1.7 μM, respectively, despite a higher administered dose in the former study. The trough levels for nordexverapamil in the same studies were 1.7 μM and 3.7 μM, respectively. Though these levels may achieve partial reversal of MDR, they are likely suboptimal for com-

plete Pgp inhibition. Nonetheless, some encouraging results have been obtained in a phase II study with lymphomas [30].

Tamoxifen is also a first generation MDR modulator whose metabolism may favour its ability to modulate Pgp. Tamoxifen concentrations of 10 μM or more are required to fully reverse MDR *in vitro* [31]. The maximum tolerated dose (MTD) is inexplicably different in two phase I studies. Trump and colleagues [32] found neurological toxicity including ataxia and dizziness to be dose limiting with an MTD of 150 mg/m$^2$ twice a day for 13 days (after a loading dose of 400 mg/m$^2$), while Millward and colleagues [33] reported nausea, vomiting and thromboembolism (in addition to dizziness) at doses exceeding 160 mg twice a day for only 6 days. Predictably, higher plasma levels were achieved in the study by Trump and coworkers [32] with mean values on days 9–13 of 4 μM for tamoxifen and 6 μM for the major metabolite of tamoxifen, *N*-desmethyltamoxifen. Similar results have been obtained by Berman and associates [34] with doses of 700 mg per day yielding serum levels of 7 μM for tamoxifen and 4 μM for *N*-desmethyltamoxifen. *In vitro* studies by Kirk and associates [31] suggest that both *N*-desmethyltamoxifen and a more minor metabolite, 4-(OH) tamoxifen are effective MDR modulators with a relative potency as follows: 4-(OH) tamoxifen (1.3) > tamoxifen (1.0) > *N*-desmethyltamoxifen (0.44). Thus, the sum of the concentrations of tamoxifen and its metabolites may reach levels in the range required for *in vitro* MDR modulation.

Cyclosporin A (CsA) is the most potent of the first generation Pgp inhibitors, with concentrations as low as 2 μM completely reversing resistance in most *in vitro* MDR models. In a large phase I study, Yahanda and colleagues [35] demonstrated that levels of 2–4 μM could be achieved with a loading dose of 6 mg/kg over 2 h followed by a continuous infusion of 18 mg/kg/day for 2.5 days (60 h). Acceptable though unpleasant toxicity consisted primarily of nausea, vomiting, fatigue, headache and confusion. Hypomagnesaemia and hypertension also occurred but were easily managed. Renal insufficiency was an infrequent but potentially severe problem and contributed to the deaths of 2 patients on study. Hyperbilirubinaemia was frequently observed when serum levels exceeded 2 μM, was always readily reversible and not associated with any measurable hepatic dysfunction. This finding, along with laboratory evidence that bilirubin is a low affinity substrate for Pgp [36] led us to postulate that hyperbilirubinaemia could be used as a marker indicating effective concentrations of modulator. However, it is now clear that bilirubin is transported predominantly by a separate anion efflux pump in the liver and that CsA is capable of inhibiting this pump as well as Pgp [37]. Interestingly, the "second generation" cyclosporin modulator PSC 833, has relatively less effect on the anion efflux pump than CsA.

When CsA was recognised as the most potent of the first generation MDR modulators, numerous cyclosporin analogues were screened for the ability to inhibit the Pgp efflux pump. PSC 833 emerged as the leading preclinical candidate with greater potency as a modulator than CsA, and no immunosuppressive activity or nephrotoxicity. Both CsA and PSC 833 undergo extensive hepatic metabolism, primarily by the P450 isoform CYP3A enzymes [38]. It is not known whether any of these metabolites have significant MDR modulating effects as is the case for verapamil and tamoxifen. Concentrations of PSC 833 as low as 0.5 μM effectively reverse MDR in some *in vitro* systems, while levels of 1–2 μM completely inhibit Pgp-mediated resistance in virtually all classical MDR cell lines. In an elegant phase I study by Boote and associates [39], PSC 833 was administered as a continuous intravenous infusion on days 2–6 with etoposide administered as a daily 2 h infusion given on days 1–5. The MTD was determined to be 10 mg/kg/day for 5 days (when given with etoposide as was done in this study) and the dose limiting toxicity was a "severe, prolonged ataxia," occurring in 2 of 9 patients at 12 mg/kg/day and both patients treated at still higher doses. Mean steady state blood levels of PSC 833 at the MTD was 2.2 μM. This particular study was unable to attribute the ataxia to the PSC 833 alone, versus a pharmacodynamic interaction between the PSC 833 and etoposide (e.g. exposing the CNS to etoposide by inhibiting the Pgp lining the blood–brain barrier). The study included a bioassay of PSC 833 activity by using serum samples from treated patients to determine the ability of such samples to increase accumulation of tritium-labelled daunorubicin by an MDR cell line. Using this *ex vivo* assay, they showed that PSC 833 serum levels of > 0.8 μM significantly increased the accumulation of daunorubicin in the Pgp-expressing cell line H69/LX4.

In subsequent phase I studies at Stanford [40, 41] an oral preparation of PSC 833 was administered alone, then subsequently with chemotherapeutic agents 2 weeks later. These studies clearly demonstrated that the ataxia initially observed by Boote and colleagues [39] is a consequence of PSC 833 alone. The ataxia is most notable at times of peak serum levels, and resolves within hours after the last dose. There is no cognitive impairment, and neurological signs include wide based gait with dysdiadochokinesia (expressed as impaired heel to shin and finger to nose coordination). The combination of PSC 833 with etoposide, paclitaxel, or doxorubicin and paclitaxel [42] has not significantly increased the incidence or severity of PSC 833 associated ataxia. In the Stanford studies, the MTD was defined as 5 mg/kg orally four times a day for up to 4 days. Doses of 6 mg/kg four times a day yielded an unacceptably high incidence of grade 3 ataxia (unable to ambulate without support, e.g. hand rail, wall, walker). Development of ataxia closely correlates with serum levels of PSC 833, with grade 3 ataxia occurring only above serum levels of 2.5 μM. Important pharmacokinetic interactions between PSC 833 and the chemotherapeutic agents will be discussed below.

## PGP EXPRESSION IN NORMAL TISSUES: POTENTIAL CONSEQUENCES

The expression of Pgp and its apical orientation in the large and small bowel, renal proximal tubules and biliary canaliculi are certainly consistent with its putative function as a xenobiotic efflux pump. Its expression along the vascular endothelium lining the central nervous system, placenta and blood–testes barrier suggest it may play a role in protecting brain, fetus and germ cells from potentially toxic or mutagenic organic cations. Functional studies of the Pgp efflux pump in the liver [43], kidney [44], intestine [45], and blood–brain barrier [46] have all demonstrated unidirectional transepithelial drug efflux of MDR related anticancer agents which is inhibited by modulators such as verapamil and CsA.

Further compelling evidence implicating endogenous Pgp as a pharmacological pump comes from studies on Pgp "knock-out" mice (see Borst and Schinkel, pages 985–990) In contrast to humans, in mice there are two genes, *mdr* 1a and *mdr* 1b, which encode P-glycoproteins that can confer

multidrug resistance [47]. Genetic engineering has permitted the breeding of mice which lack the *mdr* 1a gene [48]. The *mdr* 1a gene product is the only Pgp expressed in the mouse blood–brain barrier and is the predominant Pgp expressed in the small and large intestine of the mouse. These *mdr* 1a deficient mice appear completely healthy, yet are exceptionally sensitive to certain exogenous Pgp substrates [49]. They are 100-fold more sensitive to oral administration of the pesticide ivermectin, which has since been determined to be a Pgp substrate. Levels of this neurotoxic drug in the CNS were 100-fold higher than in normal mice expressing *mdr* 1a at the blood–brain barrier. These same mice were 3- to 4-fold more sensitive to intravenous infusion of vinblastine. Studies with double knockout mice (*mdr* 1a/*mdr* 1b -/-) are underway [48].

Recent studies have revealed an interesting effect of MDR modulators and substrates on the expression of Pgp in normal tissues. In biopsy specimens of renal allografts from patients receiving immunosuppressive doses of CsA (serum levels of 80–200 ng/ml or 0.07–0.16 μM), Pgp was overexpressed relative to normal kidney controls [50]. The same investigators showed that 7 day exposure to CsA increased expression of Pgp in the canine renal tubule cell line, MDCK. In the mouse liver, *mdr* mRNA can be increased up to 5-fold just 24 h after intravenous injection of the Pgp substrate, colchicine [51]. Cholestasis is also a potent stimulant of Pgp overexpression in the liver [52]. Thus, it appears that exposure to certain MDR related drugs can result in upregulation of Pgp expression in liver and kidney, which in turn further enhances drug elimination.

There is no evidence that normal tissue Pgp differs in structure, function, substrate specificity or affinity from the Pgp found in human tumours. The likelihood of being able to modulate tumour Pgp without having some effect on normal tissue Pgp therefore seems implausible. Possible pharmacological consequencs of normal tissue Pgp inhibition would then include (1) increased toxicity of the anticancer drugs proportional to the increase in their area under the curve (AUC), i.e. comparable to increasing the administered dose of the drug; (2) unveiling of new CNS toxicities for anticancer agents which normally are denied access to the CNS by functional Pgp; and (3) increased myelosuppression by increasing sensitivity of Pgp-expressing haematopoietic stem cells.

## PHARMACOKINETIC CONSEQUENCES OF MDR MODULATION

Most clinical studies using first generation modulators have failed to show any evidence of endogenous Pgp inhibition. Toxicities in general were those expected from the chemotherapy (and seldom more severe), as well as those anticipated from the modulator (e.g. hypotension for $Ca^{++}$ channel blockers). Numerous phase I/II studies of various designs and levels of pharmacokinetic analysis have been performed with Pgp inhibitors and the anticancer drugs, but relatively few had appropriate controls. Controlled studies are essential to understanding pharmacokinetic and pharmacodynamic drug interactions. Large interpatient variability in the disposition of anticancer drugs exists due to factors such as age, lean body mass, renal and hepatic function, plasma protein binding, concomitant medications and variations in cytochrome P450 function. Interpatient variability of anticancer drug disposition of the order of 50–100% is commonly observed and would obscure the detection of effects attributable to a modulator. Instead of an exhaustive review of uncontrolled studies, selected trials with appropriate pharmacological controls will be discussed (Table 4), highlighting the experience with second generation modulators.

Wilson and associates [28] recently published a well designed phase I pharmacokinetic study using combination chemotherapy with subsequent crossover to the same drugs plus dexverapamil. Chemotherapy consisted of cyclophosphamide (750 mg/m$^2$ i.v. bolus on day 6) and prednisone (60 mg/m$^2$ orally daily on days 1–6) and the MDR substrates etoposide (200 mg/m$^2$), doxorubicin (40 mg/m$^2$) and vincristine (1.6 mg/m$^2$) each administered as a continuous i.v. infusion given on days 1–4 (acronym EPOCH). Upon crossover, patients received the identical regimen with the addition of dexverapamil given orally at 4 h intervals on days 0–5. The dose limiting toxicity of dexverapamil was hypotension with an MTD of 150 mg/m$^2$ every 4 h. At this dose level, median trough concentrations among 19 patients were 1.1 μM (range 0.2–3.0) for dexverapamil and 1.4 μM (range 0.5–3.2) for nordexverapamil, with median peak concentrations of 1.7 μM and 1.6 μM, respectively. In 7 patients, steady state concentrations of etoposide, doxorubicin and its major metabolite doxorubicinol were assessed in separate cycles with and without dexverapamil. Though overall, the etoposide concentration at steady state ($C_{ss}$) was 10% higher in the dexverapamil group, this difference was not statistically significant. However, there was a statistically significant difference in the $C_{ss}$ for doxorubicin and doxorubicinol with nearly a 2-fold increase for both when given with dexverapamil. Nadir granulocyte and platelet counts were also lower in the dexverapamil courses, although not severe enough to require attenuation of cytotoxin doses. No differences in neuropathy or cardiac toxicity were observed.

In a smaller pharmacokinetic crossover study by Berg and colleages [53], six patients were treated with paclitaxel administered at 200 mg/m$^2$ over 3 h on day 2, with or without dexverapamil at a dose of 225 mg/m$^2$ orally every 4 h given on days 1 and 2. The crossover design permitted each of the 6 patients to serve as her own control. There was a 2-fold increase in the mean AUC and a 50% reduction in the clearance of paclitaxel in the dexverapamil arm. Changes in $Vd_{ss}$ and serum half-life ($t_{1/2}$) were not statistically significant. Grade 4 myelosuppression (nadir absolute neutrophil count $< 500/\mu l$) occurred in one of six courses without dexverapamil and in 4 of 6 courses with dexverapamil. Serum levels of the modulator were not reported, nor were levels of dexverapamil metabolites.

The cyclosporin analogue PSC 833 was studied in a phase I pharmacokinetic trial with etoposide by Boote and colleagues [39]. As mentioned above, these investigators administered etoposide as a 2 h infusion on days 1–5 with PSC 833 as a continuous infusion on days 2–6. Etoposide pharmacokinetics were assessed with the first and the fourth doses, thus allowing intrapatient comparison with and without modulator in the same treatment cycle. PSC 833 levels and toxicity were described above. Haematological toxicity required dose reduction of the etoposide (100 mg/m$^2$/day to 75 mg/m$^2$/day) as doses of PSC 833 were escalated. Among patients with PSC 833 serum levels of $\geq 1.7$ μM (2000 ng/ml), the average increase in etoposide AUC was 89% with a clearance that decreased by 46%. Furthermore, this effect appeared to plateau at PSC 833 doses above 4 mg/kg/day with no significant further increase in etoposide AUC as PSC 833 doses were

*Table 4. Effects of MDR modulators on cytotoxin drug pharmacokinetics and pharmacodynamics in controlled trials*

| Modulator | [Ref.] | Cytotoxin | Effect on cytotoxin pharmacokinetics (PK) and pharmacodynamics (PD) |
|---|---|---|---|
| Verapamil p.o. | [61] | Doxorubicin | PK: 2-fold increase in doxorubicin AUC<br>PD: Side-effect profile not reported |
| Verapamil p.o. | [62] | Epirubicin | PK: AUC: no change for epirubicin; 2-fold increase for metabolites<br>PD: No change in side-effect profile |
| Verapamil p.o. | [63] | Vindesine<br>Ifosfamide | PK: Not done<br>PD: Increase in peripheral neuropathy and constipation |
| Nifedipine p.o. | [64] | Vincristine | PK: 3-fold increase in vincristine AUC<br>PD: No change in side-effect profile |
| Nifedipine p.o. | [65] | Etoposide | PK: No change in etoposide AUC<br>PD: No change in side-effect profile |
| Dexverapamil p.o. | [53] | Paclitaxel | PK: 2-fold increase in paclitaxel AUC<br>PD: Increased neutropenia |
| Dexverapamil p.o. | [28] | EPOCH* | PK: No change in $C_{ss}$ of etoposide; ~ 2-fold increase in $C_{ss}$ for doxorubicin and doxorubicinol<br>PD: Median ANC 42% lower when modulator added |
| Cyclosporine A i.v. | [66] | Etoposide | PK: 1.8-fold increase in etoposide AUC<br>PD: Hyperbilirubinaemia, leucopenia, emesis; 50% dose reduction |
| Cyclosporine A i.v. | [56] | Doxorubicin | PK: Increase of AUC: 73% for doxorubicin; 285% for doxorubicinol<br>PD: Hyperbilirubinaemia, neutropenia, nausea and vomiting; 40–50% dose reduction with modulator |
| Cyclosporine A i.v. | [67] | Paclitaxel | PK: 50% increase in paclitaxel end of infusion concentration<br>PD: Hyperbilirubinaemia, leucopenia; 50% dose reduction with modulator |
| PSC 833 p.o. | [40] | Etoposide | PK: 2.2-fold increase in etoposide AUC<br>PD: Hyperbilirubinaemia, leucopenia; 50–60% dose reduction |
| PSC 833 i.v. | [39] | Etoposide | PK: 89% increase in etoposide AUC (PSC concentration > 2000 ng/ml)<br>PD: Hyperbilirubinaemia, leucopenia: 25% dose reduction |
| PSC 833 p.o. | [41] | Paclitaxel | PK: Not available<br>PD: Hyperbilirubinaemia, leucopenia; 50–60% dose reduction |
| PSC 833 p.o. | [42] | Paclitaxel and doxorubicin | PK: Not available<br>PD: Leucopenia; approximately 50% dose reduction |
| PSC 833 i.v. | [68] | Doxorubicin | PK: Increase in AUC: 2-fold for doxorubicin; 3.8-fold for doxorubicinol<br>PD: Similar neutrophil count with 40% dose reduction of doxorubicin |
| PSC 833 p.o. | [69] | Doxorubicin | PK: Increase in AUC: 20% for doxorubicin; 240% for doxorubicinol<br>PD: Increased leucopenia, thrombocytopenia, stomatitis; 30% dose reduction of doxorubicin |

* Serum levels of modulator are mean trough or steady state when available. p.o., oral; i.v., intravenous; EPOCH, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin; $C_{ss}$, steady state concentration; AUC, area under the curve; ANC, absolute neutrophil count. Reproduced by permission of W.B. Saunders Company from Lum B and Gosland M, *Hematol Oncol Clin North Am* 1995, Vol. 9, pp. 319–336.

escalated to 12 mg/kg/day. The design of the study did not permit controlled comparison of pharmacodynamic parameters, but the required dose reduction for neutropenia suggests that the addition of PSC 833 may have contributed to increased toxicity.

Several phase I studies with PSC 833 have been conducted at Stanford [40, 41]. There, the preferred phase I study design involves three separate but sequential treatment "periods" each with its own pharmacokinetic sampling and toxicity assessment. Individual patients are treated with the chemotherapeutic agent(s) alone (period 1), followed in three weeks by treatment with the modulator alone (period 2). Two weeks later, the patient then receives the combination of modulator and chemotherapy with significant dose reduction of the chemotherapy (period 3). Doses of modulator are escalated in cohorts of three and once the MTD of the modulator is defined, doses of chemotherapy in period 3 are escalated in subsequent cohorts. Though logistically cumbersome, this design permits comparison of toxicity and pharmacokinetics of each agent alone and in combination. Concurrent phase I studies utilising this design have been performed for PSC 833 with etoposide [40] and with paclitaxel [41]. The effect of PSC 833 on etoposide pharmacokinetics was dramatic. The area under the curve (AUC) of etoposide combined with PSC 833 (corrected for dose reduction) increased 2.2 fold when compared with the AUC of etoposide given alone. Etoposide clearance decreased by 57% when combined with PSC 833 and the serum $t_{1/2}$ increased by 57%. Pharmacodynamic parameters in both etoposide and paclitaxel trials were remarkably similar, with a dose reduction of the chemotherapy of 50–60% yielding equivalent levels of myelosuppression when compared to full dose chemotherapy alone. Non-haematological toxicity was essentially similar with the exception of toxicities peculiar to the modulator (ataxia, hyperbilirubinaemia, transient peripheral neuropathy).

As mentioned above, this study design revealed that the

ataxia noted in prior PSC 833 studies was a consequence of the PSC 833 alone and not the result of pharmacodynamic interactions with the chemotherapy. Furthermore, concern over potential exacerbation of pre-existing neuropathy (e.g. prior cispaltin exposure) was not borne out, nor was there any persistence of ataxia after PSC 833 administration was completed. Although unlikely, it remains a possibility that the ataxia is related to Pgp inhibition. Ataxia and dizziness have been noted with high doses of other modulators such as tamoxifen [32], and dexniguldipine [54]. The phase I studies with PSC 833 suggest that paclitaxel, doxorubicin and etoposide do not exacerbate this neurotoxicity and therefore either do not gain access to the CNS or alternatively, are not toxic to the CNS at the doses used.

An additional finding from the PSC 833 and paclitaxel study is that effects on bone marrow were largely limited to granulocytopenia, just as is the case for treatment with paclitaxel alone. Furthermore, there was no apparent increase in delay of count recovery. These findings alleviate some of the concern regarding Pgp expression on haematopoietic stem cells [55]. PSC 833 apparently does not sensitise marrow stem cells since pancytopenia and/or prolonged nadirs in blood counts have not been observed as a result of combined paclitaxel and PSC 833 treatment. Furthermore, our studies with CsA or PSC 833 with a variety of MDR related cytotoxins (etoposide, doxorubicin, mitoxantrone and paclitaxel) have demonstrated that the time to recovery of blood counts is not increased [35, 40–42, 56, 57].

Thus, there is substantial evidence that potent modulators can significantly alter the disposition and elimination of MDR related anticancer drugs (see Table 4). Many of the anticancer MDR substrates are metabolised by the cytochrome P450 system and excreted into the bile (as with doxorubicin, paclitaxel and vinblastine) or eliminated through both biliary and renal routes (as with etoposide). Since modulators of MDR often have a similar pharmacological fate, effects on kinetics could occur either at the level of Pgp inhibition or P450 effects, or both. These drug interactions should be considered in the design of phase II and III studies of MDR modulators and may require dose reduction of MDR related cytotoxic drugs.

## CONCLUSION

P-glycoprotein has probably played a pivotal role in the evolution of pharmacological defence mechanisms, protecting organisms from the cytotoxic and mutagenic effects [58] of a vast array of ingested xenobiotics. As we continue to search among natural products for novel compounds with anticancer activity, we will probably continue to learn more about shared mechanisms of tumour and host resistance. Predictably then, approaches to overcome resistance may result in serious challenges to the host's natural defences. Trials of new, more potent resistance modulators should be designed with caution and with careful consideration for potential pharmacokinetic and pharmacodynamic interactions [59].


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