Immunosuppressors and reversion of multidrug-resistance

Nassera Aouali a, Lahcen Eddabra b, Jérôme Macadré b, Hamid Morjani b,∗

a Roswell Park Cancer Institute, Department of Cancer Genetics, Elm and Carlton Streets, Buffalo, NY 14263, USA
b JE2428 Onco-Pharmacologie, UFR Pharmacie, IFR53, 51096 Reims, France

Abstract

Drug resistance is the major reason for failure of cancer therapy. When one drug elicits a response in tumour cells resulting in resistance to a large variety of chemically unrelated drugs, this is called multidrug-resistance (MDR). ATP-binding cassette (ABC) transporters contribute to drug resistance via ATP-dependent drug efflux. P-glycoprotein (Pgp) encoded by MDR1 gene, confers resistance to certain anticancer agents. The development of agents able to modulate MDR mediated by Pgp and ABC transporters remained a major goal for the past 10 years. Immunosuppressors, cyclosporin A (CSA) in particular, were shown to modulate Pgp activity in laboratory models and entered very early into clinical trials for reversal of MDR. The proof of reversing activity of CSA was found in phase II studies with myeloma and acute leukaemia. In phase III studies, the results were less convincing regarding the response rate, progression-free survival and overall survival were detected in advanced refractory myeloma. The non-immunosuppressive derivative PSC833 was then extensively studied. This compound shows 10-fold higher potency in reversal of MDR mediated by Pgp. Results from clinical trials with this modulator are still emerging and the notable finding was the need to reduce the dose of anticancer agent used in combination with it. Other effects of CSA and PSC833 on MDR have been described. These two molecules have been shown to have an action on the metabolism of ceramide which stands as second messenger of anticancer agents-induced apoptosis. PSC833 stimulates de novo ceramide synthesis and enhances cell death induced by anticancer agents, such as camptothecins and anthracyclines. In addition, ceramide glycosylation and storage in some cell lines have been described to play a crucial role in resistance to anticancer drugs. CSA is able to inhibit ceramide glucosylation and modulate MDR phenotype. The emergence of other modulators with several ABC protein targets like VX710 are of clinical interest in malignancies expressing several efflux pumps.

© 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: Immunosuppressors; Reversion; Multidrug-resistance

1. Introduction

Drug resistance is the major reason for failure of cancer therapy. When one drug elicits a response in tumour
cells resulting in resistance to a large variety of chemically unrelated drugs, this is called multidrug-resistance (MDR). ATP-binding cassette (ABC) transporters contribute to drug resistance via ATP-dependent drug efflux.

A diverse array of drugs have been identified that sensitize multidrug-resistant cells to chemotherapy. The drugs selected for the initial clinical studies were ones already approved for clinical use and modulators, such as verapamil, immunosuppressors (cyclosporin A), tamoxifen and quinidine were the agents most frequently evaluated. Plasma concentrations equivalent to the concentrations necessary to inhibit drug efflux in vitro were difficult or impossible to achieve with these drugs because of toxicity. Early on, it became clear that reversing the resistance of malignancies like renal cell cancer and colorectal cancer would not be possible, despite high levels of expression of ABC transporters in these tumours. It also became clear that modulators block normal excretory function and delay clearance of chemotherapy.

The limitation of the potency of the modulators has been addressed by the development of compounds that are less toxic and more effective as inhibitors. These second-generation modulators include the cyclosporin analogue PSC833 and novel agents, such as VX710. In this issue, we propose to review ABC protein-mediated multidrug-resistance and the interaction of immunosuppressors with especially P-glycoprotein-mediated drug efflux and with ceramide metabolism (an important second messenger of cell death).

2. Immunosuppressors and modulation of P-glycoprotein activity

Clinical resistance to anticancer drugs is the major reason for treatment failure. Generally, tumour cells initially respond well to chemotherapeutic agents. However, repeated drug administration often results in the selection of drug-resistant cells and hence in incurable relapses. Cancer cells can develop multiple mechanisms to evade drug toxicity [1,2]. When one drug elicits a response in tumour cells resulting in resistance to a large variety of chemically unrelated drugs, this is called multidrug-resistance. Many molecular mechanisms responsible for MDR have been discovered [3]. One group of mechanisms results in a decreased intracellular concentration and cytosolic levels of expression of ABC transporters in these tumours.

2.1. ABC transporters and substrate specificity

The three major MDR proteins are highly promiscuous transporters, they share the ability of recognizing and translocating a large number of structurally diverse, mainly hydrophobic compounds. In addition to their overlapping substrate specificity, each transporter can handle unique compounds. Pgp is a transporter for large hydrophobic, either uncharged or slightly positively charged compounds while the MRP family primarily transports hydrophobic anionic conjugates and extrudes hydrophobic uncharged drugs. The MRP1-related uncharged drug transport is linked to the transmembrane domains and one NBD on the amino-terminal side. This half-transporter is thought to homodimerize or heterodimerize to function.

Fig. 1. Structures of Pgp, MRP1 and BCRP proteins. The structures of three categories of ABC transporter. Pgp has 12 transmembrane domains (TM) and two ATP-binding sites (NBD). MRP1 is similar to Pgp in that they possess two NBD. MRP1 also contains an additional domain that is composed of five transmembrane segments at the amino-terminal end (TMD0), giving to this protein a total of 17 transmembrane domains. TMD0 is connected to the core by a cytoplasmic linker (L0). The "half-transporter" BCRP contains six transmembrane domains and one NBD on the amino-terminal side.
port or allosteric effect of cellular-free reduced glutathione [8]. The exact spectrum of the BCRP (MXR) transported sub-
strates has not yet been explored in detail and these studies
are complicated by the variable substrate-mutants of BCRP
observed in the most recent studies [12]. Some of the key
molecules are presented in Fig. 2 and are unfortunately also
MDR substrates for the patients.

Drug resistance was first documented experimentally
in mouse leukaemia cells that acquired resistance to
4-amino-N10-methylpteroylglutamic acid [13]. In 1973,
Dano discovered active outward transport of daunoru-
bicin by drug-resistant cells that were cross-resistant
to other chemotherapeutic agents, such as vinca-alkaloids
(vincristine and vinblastine) and other anthracyclines
(doxorubicin) [14]. Other authors studying MDR phenotype
noted a constant over-expression of a 170 kDa membrane
protein termed P-glycoprotein [6]. The gene encoding Pgp
was cloned and identified as MDR1 [15]. Riordan and Ling
purified also this protein from plasma membrane vesicles of
Chinese hamster ovary cell mutants with reduced colchicine
permeability [16]. More later, taxotere and taxol, other
molecule from the cytoskeleton poisons group, have been
identified as exclusive substrates of Pgp [17,18] (Fig. 2).

2.2. Agents that modulate P-glycoprotein activity

Fig. 3 shows the MDR modulators used experimentally
or in clinical trials. As early as 1987, results from the
first clinical trial of MDR (mediated by Pgp) reversal
in ovarian cancer with verapamil (VPL) in combination
with doxorubicin (DOX) were published [19]. Because of
significant cardiac toxicity, the study was discontinued. In
myeloma and non-Hodgkin’s lymphoma, VPL was clearly
shown to be active on resistance to chemotherapy protocols
with anthracyclines and/or vincaalkaloids [20,21].

Quinidine entered very early in a randomized trial in breast
cancer, aimed at reversing anthracycline resistance [22].
Quinine is much less toxic than quinidine and can be used at
higher doses. In a phase II study, Solary et al. showed that its
association with mitoxantrone and cytarabine could improve
the response rate of acute leukemias with poor prognosis
[23]. Bennis et al. showed that quinine probably modulates
resistance without any increase in intracellular accumulation
of DOX and concluded that quinine was able to alter nuclear-
and cytoplasmic distribution since the target of the anthracycline
is the nucleus [24]. More later, Belhoussine et al. showed that
modulation of resistance by quinine was not accompanied
by an increase in nuclear accumulation of pirarubicin
[25].

Biricodar (VX710), from the third-generation drugs, has
been shown to reverse MDR in vitro and in vivo by acting
on both Pgp and MRPl (Fig. 3) [26,27]. This modulator was
studied in two phase I trials in combination with DOX [28].
S9788 has a selective and high effect on Pgp transport activity
in vitro and in vivo [29] but has not been further developed
[30]. Elacridar (GG918) is active on Pgp and another ABC
protein BCRP (MXR) (Figs. 1 and 3). This compound is not
active on MRPl. Zosuquidar (LY335979) is also an active
inhibitor of Pgp in vitro and in vivo [31]. Phase I results were
recently reported showing some risk of neurotoxicity at high
dosage and no pharmacokinetic interaction with doxorubicin.
2.3. Cyclosporin A and PSC833 modulate P-glycoprotein transport activity

Cyclosporin A (CSA) and PSC833 (Fig. 4) belong to the group of MDR modulators that are able to inhibit the Pgp associated ATPase activity. The best characterized is PSC833, which at nanomolar concentrations inhibits the ATPase and transport function of Pgp. Concerning the transport of CSA and PSC833, data are not clear. Several works favor the view that CSA and PSC833 are transport substrates for Pgp [34–36] and other data do not [37]. The last report considers that PSC833 is indeed a substrate of Pgp, but a slow one. That is, when PSC833 competes with a substrate, it will win out due to its higher affinity (a larger interaction surface) [38]. But since its transport rate is slower, it will slow down the turnover rate, which will then be reflected in the decrease in ATPase activity. So, PSC833 seems to be a partial antagonist, since it does not completely block Pgp function, but just slows it down due to the bulkiness of this molecule acting as an "obstructive" substrate, slowing down the Pgp machinery.

Studies performed in vitro on samples from leukaemia patients showed that CSA and PSC833 were appropriate inhibitors that can be used for MDR diagnosis. In fact, Merlin et al. have shown that in the Pgp-positive samples, a significant increase in cellular daunorubicin accumulation is observed in the presence of CSA [39]. More later, the same group demonstrated the influence of PSC833 on daunorubicin intracellular accumulation in bone marrow specimens from patients with acute myeloid leukaemia [40]. Legrand et al. have demonstrated also that there was a good correlation between Pgp expression and the in vitro modulatory effect of CSA on calcine-AM uptake in sample patients with acute myeloid leukaemia [41]. A simultaneous functional study of calcine-AM uptake and calcine efflux in the presence of, respectively, CSA and probenecid (a specific inhibitor of MRP1, Fig. 3) have shown that it was possible to discriminate the Pgp and MRP1 transport activities [42].

CSA entered early into trials of reversal of multidrug-resistance. The proof of reversing activity of CSA was found in phase II studies with myeloma [43] and acute leukaemia [44]. Phase III studies were conducted in haematological malignancies and no effect of CSA on the overall response rate and progression-free survival in myeloma patients [45], whereas among several studies, only one showed a positive effect of CSA in acute myeloblastic leukaemia [46].

A widely tested second-generation compound is PSC833 (valsopodar), a derivative of cyclosporin D that is 10 times more potent than CSA [47]. PSC833 was the first molecule from cyclosporins group without immunosuppressive properties [48] and showed modulation of MDR in vivo with a lower renal toxicity when compared with CSA [49]. During phase I studies, an important effect of this compound on the pharmacokinetics of the associated drugs was shown, the antitumour drugs being etoposide (VP16) [50], DOX [51], mitoxantrone (MIT) [52] and taxol [53]. In most cases, either a doubling of the time-plasma concentration area under the curve or an important increase in elimination half-life was found. Therefore, it was not possible to separate the pharmacokinetic effects of PSC833 from its pharmacodynamic effects. Those results were in general disappointing, particularly in acute myeloblastic leukaemia trials and then suggested that it was he needed to reduce the dose of anticancer agent used in combination with it and dose reduction ranged from 25% for etoposide to 66% for taxol [54,55]. Those dose reductions were required to prevent toxicities of the anticancer agent in combined therapy and have compromised drug concentration in the tumour even with complete inhibition of Pgp.

One treatment that has not been fully explored is that of prevention of the emergence of resistance through the use of Pgp inhibitors. In the laboratory, PSC833 reduced the mutation rate for DOX-selected resistance in sarcoma cells by 10-fold, thus reducing the development of resistant clones in vitro. In those sarcoma cells, treated with PSC833, resistance was mediated by an alternative pathway with reduced expression of topoisomerase IIα, the target enzyme for anthracyclines [56]. Another study examined six agents for their ability to prevent vincristine resistance in a rhabdomyosarcoma cell line [57]. MDR modulators and
particularly PSC833 prevented the development of resistance, suggesting the role of the use of Pgp inhibitors prior to cytotoxic therapy.

3. Immunosuppressors and ceramide metabolism

3.1. De novo synthesis of ceramide and resistance to apoptosis

Sphingolipid are a large family of lipids that are implicated in signal transduction process and ceramide is the most studied (Fig. 5) [58–60]. Ceramide synthesis results from sphingomyelin hydrolysis [58] or by de novo synthesis (Fig. 5). De novo synthesis of ceramide is initiated at the cytosolic surface of the endoplasmic reticulum (ER) membrane by condensation of L-serine and palmitoyl coenzyme A [61,62]. This NADPH-dependent reaction gives 3-ketosphinganine [63], which is reduced to dihydrosphingosine. The next step is an acylation reaction. The enzyme acyltransferase transfers a long-chain fatty acid to the amino group of the molecule and generates dihydroceramide. The introduction of a trans-4,5 double bond by a desaturase converts the dihydroceramide to ceramide (Fig. 5) [64].

A number of cytotoxic agents have been shown to activate de novo synthesis of ceramide. Daunorubicine, an inhibitor of topoisomerase II from anthracycline family is able to promote ceramide formation and apoptosis by ceramide synthase activation in P388 and U937 cells [65] and in human myeloid leukaemia cells [66]. Vinca-alkaloids which target tubulin polymerisation increases cellular ceramide. In fact, vincristine treatment of ALL-697 leukaemia cells induces apoptosis after an increase in ceramide level [67]. In CCRF-CEM leukaemia cells, vincristine treatment induces apoptosis and an elevation of ceramide [68]. Similar data have been obtained in human epidermoid carcinoma cells KB-3-44 cells [69]. Taxol, which is able to inhibit the microtubules depolymerisation, induces apoptosis in a number of cell lines including leukaemia and breast cancer. It induces ceramide production and acts synergistically with administration of exogenous ceramide [70,71].

Etoposide, which inhibits topoisomerase II, also induces an increase in ceramide level [72]. Etoposide is able to increase ceramide level via activation of serine palmitoyl-

**Fig. 5.** Ceramide metabolism pathways. PSC833 and cyclosporin A are able to inhibit de novo synthesis and glucosylation of ceramide, respectively.
transferase [73,74]. Camptothecin, a topoisomerase I inhibitor, increases the ceramide level in 4B1 mouse fibroblasts [75] and HT-29 colon cancer cells [76]. In LNCaP prostate cancer cells, camptothecin treatment induces an elevation of ceramide and apoptosis [77]. The synthetic retinoids are also to induce an increase in cellular ceramide level and apoptosis. In PCC7-Mz1 stem cells, retinoic acid is able to increase ceramide level and apoptosis in PCC7-Mz1 prostate cancer cells, the effect of 4-HPR on ceramide synthesis was shown to be through activation of serine palmitoyltransferase [80,81]. Ganciclovir is able to activate the de novo ceramide synthesis of ceramide in A549 cells [82]. Moreover, it has been shown that A9-tetrahydrocannabinol (THC) and other cannabinoids induce ceramide elevation and apoptosis in cancer cells by activating the serine palmitoyltransferase [80,81]. PSC833, in addition, at this action on Pgp is able to increase the ceramide level by a Pgp-independent mechanism [85,86]. In fact, PSC833 induces a ceramide generation associated with a decrease in cell survival in drug-resistant MCF7 cells [85]. Moreover, in KB-V-1 human epidermoid carcinoma cells, PSC833 activates ceramide synthesis and increases vinblastine sensitivity [69]. The PSC833 effect on ceramide generation has been also demonstrated in human ovarian carcinoma cells SKOV-3 [87]. Moreover, cells whose ceramide glycosylation is enhanced, are resistant to PSC833 effect [88]. This can be explained by the fact that ceramide generated by PSC833 is converted to glucosylceramide [89]. Co-treatment with PSC833 and fumonisin B1 (a ceramide synthase inhibitor) indicates that PSC833 activates the de novo synthesis of ceramide in cancer cells [69,85,88]. Recently, a study on MDA-MB 468 breast cancer cells has shown that generation of ceramide by PSC833 results from activation of serine palmitoyltransferase enzyme [90]. In addition, PSC833 is able to restore an sphingomyelin-ceramide pathway stimulation. In fact, the amount of hydrolysable sphingomyelin is another candidate for regulating ceramide production and sphingomyelin is distributed on both inner and outer leaflets of the plasma membrane [91]. Bezombes et al. have reported that the KGLa cells, which are inherently resistant to TNFs and do not produce ceramide upon cytokine stimulation, can be sensitized by the use PSC833 [92]. The authors suggested that resistance to TNFα-mediated apoptosis of these cells is linked to the disposability of the sphingomyelin pool, and a role for P-glycoprotein in sphingomyelin transverse plasma membrane asymmetry which can be affected in this case by PSC833.

3.2. Ceramide glycosylation and multidrug-resistance

Once generated, ceramide can accumulate in the cell or may be converted into a variety of metabolites. In fact, ceramide is a precursor of both glycosphingolipids and sphingomyelin (Fig. 5). The transfer of phosphocholine group of phosphatidylcholine to ceramide generates sphingomyelin. The glycosylceramide (GlcCer) synthesis results from transfer of glucose of UDP-glucose to ceramide by a glucosyltransferase (Fig. 5). The GlcCer is a metabolic precursor of lactosylceramide (LacCer) and gangliosides. Early reports already indicate that the lipid composition of drug-resistant cells is different from that of sensitive cells [93]. Lavie et al. have shown that MDR cells, which over-express Pgp, display an elevation of GlcCer when compared to the drug-sensitive counterparts [94–96]. Kok et al. have reported that colchicine-resistant colon carcinoma cells over-expressing MRP1 have elevated GlcCer level [97]. It has then been suggested to consider GlcCer level as a diagnosis marker for drug resistance in tumours [98].

The increase in GlcCer level has been explained by a higher activity of GlcCer synthase (GCS) [95] and/or uncoupling of GlcCer conversion to LacCer [99]. Several arguments are in favor of a role for GCS in MDR, including the observation that over-expression of GCS confers resistance to chemotherapy [95,100] and on the other hand, transfection of multidrug-resistant cells with a GCS antisense oligonucleotide attenuates resistance [101]. However, a recent study indicates that the absence of functional GCS does not sensitize melanoma cells to anticancer drugs [102].

The inhibition of ceramide glycosylation increases sensitivity of MDR cells to chemotherapeutic agents [69]. Moreover, the use of specific glycosylceramide synthase inhibitors like 1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP) decreases the GlcCer level in resistant cells and partially restores sensitivity to chemotherapeutic agents [96,103–105].

Cyclosporin A, that is used in first for treatment of estrogen receptor positive breast cancer [106], is able to inhibit the GlcCer synthesis in MCF7/ADR which are oestrogen receptor negative and hence modulate their resistance to chemotherapy [107,103]. This has been also shown in other cell lines [96,108]. However, this mechanism is not well known [103].

Cyclosporin A is able to block the glycosylation of ceramide. In fact, a study on MCF7/ADR cells has shown that cyclosporin A is a potent inhibitor of glucosylceramide synthase [88]. The effect of cyclosporin A on ceramide glycosylation is accompanied by a circumvention of MDR phenotype [103]. The same data have been reported by Morjani et al. [96]. However, the mechanism by which cyclosporin A inhibits ceramide glycosylation is inhibited is not clear.

4. Conclusion

A number of lessons have been learned from the evolution of the field of multidrug-resistance. A very nice and recent study have well demonstrated that prediction of drug sensitivity and resistance can be accessed by profiling ABC transporter genes in cancer cells [109]. However, the reversal
of multidrug-resistance has not yet reached the level of routine clinical applications. The future of the potential therapeu- tic area remains uncertain. This is not for lack of molecules, since hundreds of compounds have been selected or designed with comprehensive studies on structure–activity relationships in several chemical families. Rather, the reason for this failure originates from the inadequate design of clinical trials. The pharmacological and toxicological properties of multidrug-resistance modulators should have been taken into consideration with those of anticancer drugs in terms of the benefits/cost ratio for the patient.

The pharmacokinetic interaction of cyclosporin A or PSC833 with cytotoxic drugs is considerably rendered difficult the clinical development of these drugs as multidrug-resistance modulators. In fact, cyclosporin A and PSC833 appear to be the only ones to present this interaction that might well be related to the specific inhibition of another ABC protein that P-glycoprotein involved in the biliary elimination of drugs. Another property of PSC833 has been reported in a recent work by Lehn et al. who have shown in vivo that a simple inhibition of P-glycoprotein by PSC833 may lead to direct elimination of multidrug-resistant cells due to the fact that this membrane protein is involved in malignancy as well as in drug resistance [110]. Concerning ABC transporters, one emerging idea is the use of inhibitors in enhancing the oral bioavailability of anticancer drugs.

Reviewers
Dr. Marc Diederich, Fondation Recherche sur le Cancer et les Maladies du Sang, Laboratoire de Biologie Moléculaire et Cellulaire, du Cancer (LBMC), Hôpital Kirchberg, 9, rue Edward Steichen, L-2540 Luxembourg.

Prof. Jean-Louis Merlin, Laboratoire de Recherche en Oncologie, Centre Alexis Vautrin, Av. de Bourgogne, F-54511 Vandoeuvre-Les Nancy Cedex, France.

Prof. Xavier Ronot, Directeur du Laboratoire de Dynamique Cellulaire de l’EPHE UMR CNRS 5525, In3S, Université Joseph Fourier, F-38706 La Tronche Cedex, France.

References


[28] Penn RA, Hewitt J, Harding MW, et al. Phase I and pharma-


[37] Stein WD. Kinetics of the multidrug transporter (P-glycoprotein) and its reversal. Physiol Rev 1997;77:S45–60.


[48] Boettje DJ, Pettey PR, Twentyman PR, et al. Phase I study of topo-


[50] Twentyman PR, Aarts HI, Tallman MS, et al. Treatment of refractory and relapsed acute myelogenous leukemia with combination chemother-


[52] Cocker HA, Tiffin N, Pritchard-Jones K, Pinkerton CR, Kelland LR. In vitro prevention of the emergence of multidrug resis-

[53] Cooker HA, Tiffin N, Pritchard-Jones K, Pinkerton CR, Kelland LR. In vitro prevention of the emergence of multidrug resis-


Biography

Hamid Morjani is 41 years old and obtained his Ph.D. degree in Biophysics in 1993. He became lecturer researcher in the University of Reims, France in 1993, where he is currently working in the JE2428 Onco-Pharmacology Group. He is a member of the American Association for Cancer Research and the Federation of European Biochemical Societies. He has authored 40 articles, mainly in the field of “multidrug-resistance to anticancer drugs” and especially on “mechanisms of resistance to topoisomerase I and II inhibitors, and telomerase inhibitors belonging to the G-quadruplex ligand group”.