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SDZ 280-125: a cyclopeptolide endowed with an *in vitro* cyclosporin A-like profile of activity for the reversion of the P-glycoprotein-mediated multidrug resistance of tumor cells

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Tumor cells whose multidrug resistance is caused by the P-glycoprotein (Pgp) mediated anti-cancer drug (ACD) efflux can be chemosensitized by cyclosporins, whose derivatives were found to display a whole range of resistance-modulating activities. Similarly, derivatives of the non-immunosuppressive natural fungus cyclic peptolide SDZ 90-215 were recently shown to display a broad range of chemosensitizing activities. With highly resistant cells expressing high levels of Pgp, one such compound (SDZ 280-125) was shown here to restore both a normal sensitivity to the growth-inhibitory effects of ACD and a normal retention of an anthracycline antibiotic. With both read-outs, SDZ 280-125 activity was about 3-fold that of cyclosporin A (CsA). SDZ 280-125 also displayed the same profile of chemosensitization as CsA for different ACD classes.

Key words: Multidrug resistance, P-glycoprotein, resistance-modulating agents.

Introduction

The emergence of tumor cells with a multidrug-resistance (MDR) phenotype is one of the major problems that clinicians have to face during cancer treatment by chemotherapy. One mechanism responsible for this pleiotropic resistance is the overexpression of the 170 000 kDa transmembranous P-glycoprotein (Pgp). This protein probably functions as an energy-dependent pump and maintains the drug steady-state level inside the cell below its active threshold.[1,2]

Several experimental systems with different MDR sublines of cancer cells have been developed to understand this mechanism of resistance. They have permitted the identification of very diverse compounds, such as calcium channel blockers, cepharantines, chloroquine, isoprenoid, progesterone and vitamin A, all of which can restore some sensitivity of MDR cells to anti-cancer drugs (ACD). All these chemosensitizers, or resistance-modulating (RM-) agents (RMA), have different functions and structures, but they share hydrophobicity.[1,2]

Another class of hydrophobic molecules known to down-modulate MDR are cyclic undecapeptides: the set of cyclosporins and their derivatives.[3–6] Particularly, a photoaffinity derivative of cyclosporin A (CsA) was shown to be able to bind Pgp[7] and CsA could restore drug retention in Pgp-expressing cells.[8] Among a series of RMAs compared *in vitro* for their capacity to restore a normal ACD sensitivity (normal IC_{50} range) to the MDR cells, CsA was shown to be more active than other RMAs, including some already used in early clinical trials.[5,9] However, CsA had been selected for its immunosuppressive (IS) properties, which might not be adequate for adjuvant chemotherapy of cancer patients. Within the cyclosporin family, a large number of variants with widely different RM activities were identified, in particular cyclosporins devoid of IS activity but endowed with RM activity.[3,4] One of these derivatives, SDZ PSC 833,[6,8,10] recently entered early clinical trials.

Since our screening of cyclosporin derivatives extended to several hundred different molecules, a fair relationship could be inferred between structural features and RM activities. Particularly, besides the aforementioned hydrophobiticy, the cyclic character of the peptide seemed to be an essential feature for RM activity (Loor *et al.*, unpublished data). A variety of natural cyclic peptides with sizes and gross chemical features similar to cyclosporins were thus assayed for RM activity (Loor *et al.*, unpub-

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lished data), but only one of them looked interesting. It was a natural cyclopeptolide isolated from the fermentation broth of a Fungus imperfecti (*Septoria* sp., NRRL 15761). The native form of this pipecolic acid-containing cyclopeptide displayed virtually no RM activity, but chemical modification could endow the cyclopeptolide with very high RM activity such as shown by its previously described derivative SDZ 280-446 which is as potent as SDZ PSC 833.[11–14] Moreover, the profiles of chemosensitization were similar for SDZ 280-446 and for SDZ PSC 833 (as well as CsA). That is, no qualitative differences such as a preferential chemosensitization for (a) particular structural ACD class(es) were found. This was at variance with the recent observation that non-IS derivatives of FK506 tended to give higher gains of sensitivity to doxorubicin (DOX) than to colchicine (COL).[15]

In the course of studies designed to establish the structure–activity relationships for this cyclopeptolide family, a strong feature emerged:[16] in all cyclopeptolide tested, the isomerization of D-Lact^{10} into L-Lact^{10} always brought a marked increase of RM activity, SDZ 280-446 belonging to the L-Lact^{10} class. Nevertheless, the D-Lact^{10} forms are more easily accessible derivatives of the naturally occurring cyclopeptolide and therefore are not devoid of interest. Whether both classes of isomers share the same general mode of action on resistance modulation and the same spectrum of activity in inhibiting Pgp molecules was, however, unknown—the structure–activity relationships being essentially restricted to the use of colchicine as Pgp substrate.[16] Particularly, the large structural alterations caused by the D-Lact^{10} to L-Lact^{10} isomerization might alter the interaction of the cyclopeptolide with the Pgp-using MDR pump in such ways that the differences of RM activity might not only be quantitative, but also qualitative, i.e. change the resistance spectrum differentially.

Because their relative strengths are an order of magnitude different, qualitative comparisons of L-Lact^{10}-containing isomers with their D-Lact^{10}-containing congeners are not straightforward. Indeed, very different concentrations of isomers are needed to reach the same approximate level of specific inhibition of Pgp function. This implies widely different impacts of unspecific effects of the compounds on the cell plasma membrane, as can be expected for highly hydrophobic molecules. Therefore, one D-Lact^{10}-containing cyclopeptolide, SDZ 280-125, which displayed levels of RM activity comparable to that of CsA, was selected for this study of the drug-sensitivity restoration profile and of the capacity to restore daunomycin (DAU) retention in MDR tumor cells.

Materials and methods

Drugs

COL (Sandoz Pharma, Basel, Switzerland), vincristine (VCR; vincristine sulfate; Serva Feinbiochemica, Heidelberg, Germany) and DAU (Sigma, St Louis, MO) were prepared as stock solutions (1 mg/ml) in 0.9% sodium chloride, stored at +4°C and used within 1 month, whereas etoposide (VP-16, Sandoz) was prepared in dimethylsulfoxide (DMSO). CsA (Sandoz) and SDZ 280-125 (Sandoz) were prepared as stock solutions in absolute ethanol.

The final concentrations of ethanol and DMSO in the cell culture were never larger than 0.3 and 0.1%, respectively, which are non-toxic to the cells.

Tumor cell lines

The pairs of parental (Par) and multidrug resistant (MDR) cell lines belonging to three species and three cell classes covering all levels of adherence from none to very strong: the murine monocytic leukemia P388 and a DOX-resistant subline (P388R) obtained from Dr M Grandi (Farmitalia, C. Erba Research Center, Milano, Italy), the chinese hamster ovary (CHO) fibroblastoid carcinoma AUX B1 (subline ABISII) and its MDR mutant CH^{R}C5 (subclone C5S3.2) obtained from Dr V Ling (Ontario Cancer Institute, Toronto, Ontario, Canada) and the human nasopharyngeal KB carcinoma (KB-3-1) and its MDR clone (KBV.1) obtained from Dr I Pastan (NCI, NIH, Bethesda, MA).

All MDR cell lines were continuously grown in the presence of the drug used for their selection; 8–24 h before each experiment the culture medium of the MDR cell lines was removed and the cells were grown in drug-free medium. The detailed conditions for *in vitro* growth and analyses of these six different cell lines were as published earlier.[6]

Determination of chemosensitization activity *in vitro*

Tumor cell growth and its drug-mediated inhibition were measured as described previously.[4–6] Briefly, cells were cultured for a few days (37°C, 7% CO_2) in the presence of a whole range of ACD concentra-

tions (variable depending on the ACD) and a whole range of RMA concentrations (0.025–10 μM). Then, an MTT assay was performed and the absorbances were read at 540 nm. The duration of culture and cell numbers at the beginning of the culture were determined such that the cells would still be in the exponential growth phase at the time of MTT addition.

The growth levels obtained without RMA and ACD but with their solvents were taken as 100% growth. The ACD IC_{50}s were calculated from the dose–response curves obtained by plotting the measured growth versus the ACD concentration as described previously.[13] Cultures performed in the absence of ACD (but in the presence of its solvent) with the whole range of RMA concentrations allowed the construction of RMA dose–cell growth response curves and the determination of the RMA IC_{50}s.[5,6] In chemosensitization assays, only RMA concentrations giving less than 10–20% growth inhibition of the particular cell line were considered to give significant results.

A complete ACD dose–cell growth response curve was constructed at each RMA concentration. A whole range of 'IC_{50}^{+}' values were thus obtained in the *presence* of the whole range of RMA concentrations, the 'IC_{50}^{-}' values being obtained in the *absence* of RMA (but in the presence of its solvent). The increases of ACD sensitivity or 'gains' in sensitivity of the RMA-treated cells were given by the ratio IC_{50}^{-}/IC_{50}^{+} and a gain was calculated for each RMA concentration.[6]

Intracellular fluorescence studies for DAU retention

The method was adapted for 96-well microplates from our former procedure.[8] Briefly, samples of 5×10^5 cells were incubated in a 7% CO_2 humidified atmosphere at 37°C for 30 min in 0.2 ml medium containing 20 μM DAU in the absence or presence of RMA. The DAU excess not taken up or not retained by the cells was removed by three washes (2 min centrifugation at 200 *g* and 4°C) and the cells were reincubated for 15 min at 37°C in drug-free medium (lacking both DAU and RMA). After two further washes by centrifugation in DAU- and RMA-free medium, the cells were fixed in 1 ml of PBS–3.7% formaldehyde and analyzed for intracellular DAU fluorescence with a FACScan cell analyzer (Becton-Dickinson, Mountain View, CA) equipped with an argon laser (15 mW) tuned at 488 nm. Dead cells and debris were excluded by setting a gate on the basis of their decreased forward light scatter.

In the fluorescence histograms, the *x*-axis was a logarithmic scale for the fluorescence level and the *y*-axis was an arithmetic scale for the number of cells recorded in each channel. In order to simplify some comparisons, some data were presented as the percentage of the geometric mean fluorescence of the MDR-P388 to the geometric mean fluorescence of the Par-P388 cells.

Results

The novel cyclopeptolide SDZ 280-125

SDZ 280-125 (Figure 1) is Cyclo[Pec-MeVal-Val-MeAsp-(ω-Val-*O*-Benzyl)-Melle-Melle-Gly-MeVal-Tyr(Me)-D-Lact] (molecular weight 1316.7). It is one of a series of semi-synthetic undecapeptolides obtained by chemical modification of SDZ 90-215, a natural cyclic peptolide isolated from an imperfect Fungus (*Septoria* sp., NRRL 15761).[16] Its lack of IS activity was confirmed in a mixed lymphocyte reactivity assay (not shown). Its RM activity was assayed with a few pairs of Par and MDR tumor cell lines for its capacity to restore the drug-sensitivity of *in vitro* proliferating MDR cells and the DAU retention by MDR-P388 cells.

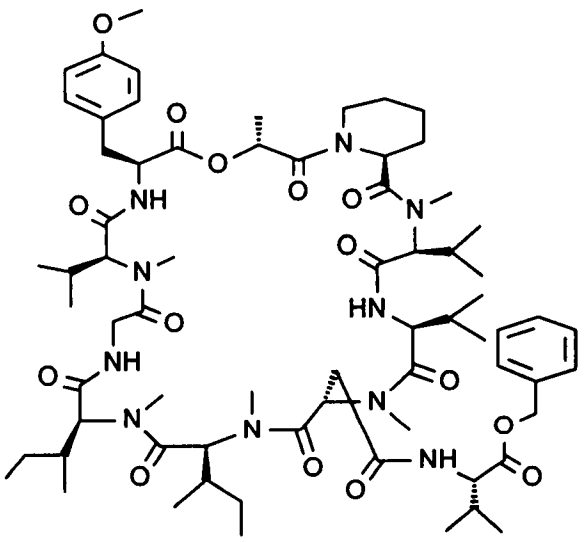

Figure 1. Structure of SDZ 280-125. It is Cyclo[Pec-MeVal-Val-MeAsp-(ω-Val-*O*-Benzyl)-Melle-Melle-Gly-MeVal-Tyr(Me)-D-Lact].

Chemosensitization of MDR tumor cells with SDZ 280-125

In preliminary experiments, significant decreases of ACD IC_{50} were already observed at 0.025 μM for low Pgp-expressing Par-CHO cells, with a maximal 10-fold ACD IC_{50} decrease in comparison with the IC_{50}s in the absence of SDZ 280-125. For the high Pgp-expressing MDR-CHO cells, important decreases of ACD IC_{50} were observed at 0.75 μM SDZ 280-125 (Table 1), but a total reversion of their high resistance required 7.5 μM SDZ 280-125 (this being, however, close to the IC_{50} of the RMA alone). A comparison with CsA is provided below.

SDZ 280-125 could also sensitize the high Pgp-expressing MDR-P388 cells (0.75 μM SDZ 280-125 increased COL-sensitivity more than 40 times) while having no effect at all on the Par-P388 cells (known to be fully ACD-sensitive and to lack Pgp expression) (Table 1). Direct cell growth inhibition by low doses of CsA[6] did not allow a comparison with SDZ 280-125.

Neither the Par-KB cells, which lack Pgp, nor the MDR-KB cells, which express extremely high amounts of Pgp and resistance levels, could be detectably sensitized by SDZ 280-125 (Table 1). However, as shown earlier,[6] the RM activity of CsA was also insufficient to chemosensitize those MDR-KB cells.

Table 1. Comparison of some gains of sensitivity for MDR tumor cells obtained by SDZ 280-125 concentrations at least 10-fold lower than the IC_{50} of the RMA alone.

ACD	Cells	Gains[a] obtained with SDZ 280-125 at		
		0.075 μM	0.25 μM	0.75μM
VP-16	Par-CHO	2.9 ± 2.5	6.6 ± 1.0	7.8 ± 0.7
	MDR-CHO	1.9 ± 0.2	12.8 ± 2.1	49.5 ± 17.7
VCR	Par-CHO	3.6 ± 0.5	9.8 ± 0.0	10.6 ± 0.2
	MDR-CHO	2.4 ± 0.1	18.9 ± 2.9	83.9 ± 2.6
DAU	Par-CHO	4.1 ± 0.1	6.8 ± 1.2	8.6 ± 1.4
	MDR-CHO	1.4 ± 1.3	21.3 ± 4.3	91.5 ± 2.5
COL	Par-CHO	4.3 ± 0.7	10.3 ± 1.3	9.7 ± 0.2
	MDR-CHO	1.6 ± 0.4	11.8 ± 0.7	60.7 ± 22.4
COL	Par-P388	0.9 ± 0.1	ND	0.8 ± 0.1
	MDR-P388	1.3 ± 0.2	ND	44.3 ± 3.1
COL	Par-KB	1.2 ± 0.1	ND	1.3 ± 0.5
	MDR-KB	1.1 ± 0.1	ND	1.3 ± 0.0

[a]Means ± SD from independent experiments (three with CHO cells, two with P388 cells and KB cell) in triplicates.
ND = not done.

Comparison of the RM activities of CsA and SDZ 280-125 using the pair of Par-CHO and MDR-CHO cell lines

When SDZ 280-125 and CsA were compared, using the pair of low Pgp-expressing Par-CHO and high Pgp-expressing MDR-CHO cell lines, both compounds displayed similar direct growth inhibition properties: CsA was slightly more inhibitory for Par-CHO cells (70% growth inhibition by 10 μM CsA) than for MDR-CHO cells (26%), whereas the SDZ 280-125 IC_{50} for the growth of both cell lines was 7.5 μM (not shown).

Both compounds were found active on the Par-CHO cells, whatever the ACD tested. With the MDR-CHO cells, high, but non-toxic doses (2–5 μM) of both compounds led to a marked sensitization. With the exception of VP-16, a complete reversion could be obtained, i.e. the IC_{50}s of the RMA-treated MDR cells were as low as the IC_{50}s of the RMA-treated Par-MDR (fully ACD-sensitized Par-CHO cells) (Figure 2).

At lower concentrations, however, the cyclopeptolide was more active than CsA. In fact, to obtain a 10-fold reduction in resistance to COL, DAU, VP-16 and VCR, 0.5–0.7 μM CsA were needed in comparison with only about 0.2 μM SDZ 280-125. This non-IS cyclopeptolide was thus, as a mean, 3.1-fold more active than CsA for the MDR-CHO cell sensitization (precisely: 2.3 × for COL, 3.1 × for DAU, 3.1 × for VP-16 and 3.9 × for VCR). Nevertheless, it showed the same ACD chemosensitization profile as CsA.

Restoration of DAU retention in MDR-P388 cells by SDZ 280-125

This short-term assay of Pgp function inhibition, which does not require cell growth, could be performed in comparison with CsA, as well as with the reference compound SDZ PSC 833. The intracellular DAU retention was measured by the degree of anthracycline fluorescence of the P388 cells following *in vitro* exposure to DAU. These flow cytometry analyses of DAU retention were performed with both Par and MDR cells as a function of the RMA concentration. In the absence of RMA treatment, the MDR-P388 cells displayed low fluorescence levels corresponding to 2.2 ± 0.5% of the Par-P388 cell fluorescence levels.

The presence of the tested range of RMA concentrations, during the exposure of the Par-P388 cells to DAU, had no detectable effect on the level of DAU retention, as measured by unchanged fluorescence

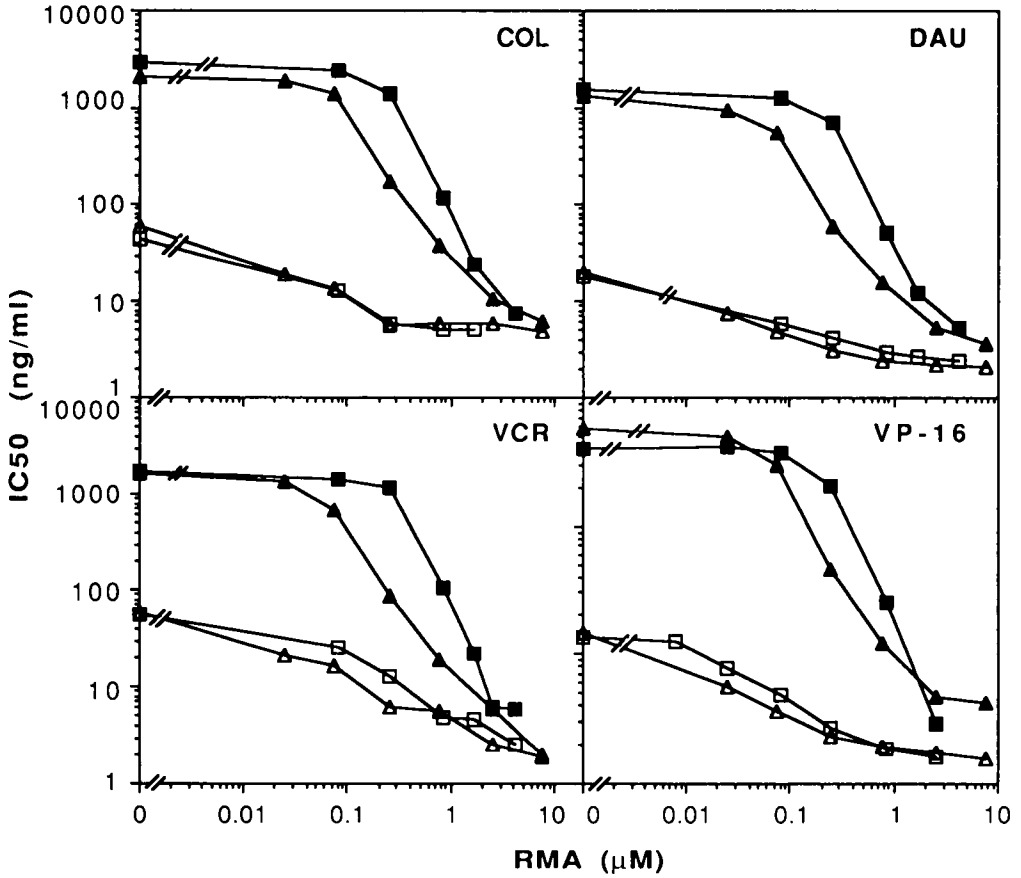

Figure 2. *In vitro* chemosensitization of Par (open symbols) and MDR (closed symbols) CHO cells by CsA (squares) and SDZ 280-125 (triangles). The ACD tested were COL, DAU, VCR and VP-16. The cell proliferation was assessed by the MTT assay and the IC_{50}s in the absence or presence of the RMA were determined (means of three to five independent experiments in triplicates). These isobolograms compare the decreases of ACD IC_{50} (*y*-axes) as a function of the increased RMA concentrations (*x*-axes), as obtained with the two RMA under study.

levels of the Par-P388 cells. On the contrary, definite shifts of the fluorescence profiles of the RMA-treated MDR-P388 cell populations were observed by treatment with either of the three RMA, though these shifts were obtained at different concentrations for the different RMA (Figure 3).

Thus, all three RMA could virtually restore the normal Par-P388 level of fluorescence in the MDR-P388 cells. Nevertheless there were wide differences of strength of the three RMA for this activity: while SDZ PSC 833, at a concentration of 0.3 μM only, could restore the DAU retention of MDR-P388 cells almost to the Par-P388 cell level, with the other two softer RMA the same approximate level of restoration required 30 μM of CsA or 10 μM of SDZ 280-125 (Table 2). Therefore, for this assay and this type of comparison, the cyclopeptolide SDZ 280-125 appeared to be about 3-fold more active than CsA, though remaining largely less active than the cyclosporin derivative SDZ PSC 833.

Discussion

The drugs tested here as Pgp-pump substrates, i.e. COL, VCR, DAU and VP-16, had widely different

Table 2. Restoration of DAU retention in MDR-P388 cells by SDZ 280-125 in comparison with CsA

RMA	μM	MDR-P388/Par-P388 fluorescence ratio[a]
CsA	3	51.8 ± 8.0
	10	77.8 ± 12.0
	30	90.2 ± 8.9
SDZ 280-125	3	61.7 ± 3.9
	10	94.0 ± 14.9
	30	103.6 ± 12.2
Ethanol solvent		2.2 ± 0.5

[a]Means ± SD from four independent experiments.

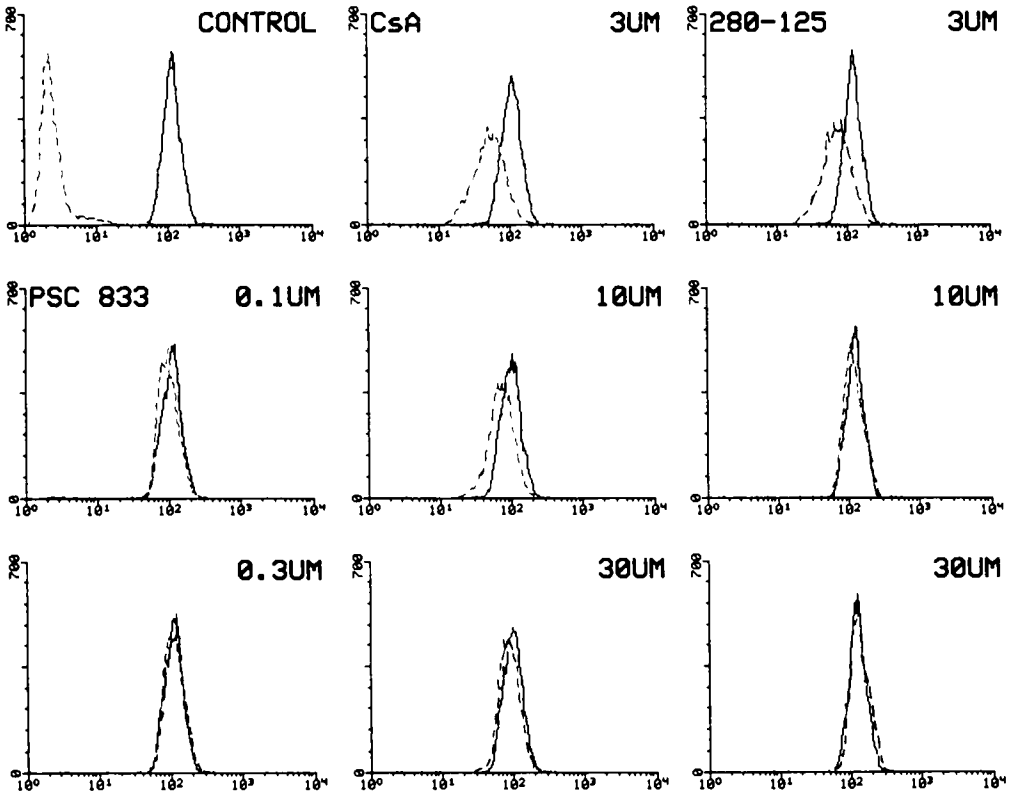

Figure 3. DAU retention by Par (—) and MDR (– – –) P388 cells. Comparison of the effects of three concentrations of SDZ 280-125 and CsA with two reference concentrations of SDZ PSC 833 and the ethanol solvent control. The experimental conditions measure the short-term persistence of the RMA-mediated inhibition of the Pgp-dependent DAU efflux: the RMA was present only during the DAU uptake phase and no longer during the DAU efflux phase. The fluorescence of individual cells was recorded on the *x*-axis as a logarithmic scale and the number of cells in each fluorescence channel was recorded on the *y*-axis. A virtually complete identity of the fluorescence profiles of the Par-P388 and MDR-P388 cell populations is achieved at 0.3 μM SDZ PSC 833, 30 μM CsA and 10 μM SDZ 280-125.

structures, possibly resulting in different Pgp-mediated efflux pathways. In spite of their broad cross-resistance to various drugs, each Pgp-expressing MDR cell line can indeed display its own pattern of resistance, with a definite preferential resistance to the drug used for selection.[1,2] Various single point mutations on the Pgp molecules were shown to be sufficient to produce very different profiles of cross-resistance,[1,17,18] suggesting that different pharmacophores might be involved for the Pgp-mediated processing of drugs such as COL, DOX and dactinomycin.

Thus, different drug binding/transport sites on the Pgp might indeed exist for different drugs or groups of drugs, and it could be expected that a given MDR cell line may also show different degrees of chemosensitization for different drugs by chemosensitizers which display widely different structures. Results of studies on the drug and RMA binding to Pgp[7,19,20] also suggest that some RMA might display preferential binding to the different hypothetical ACD pharmacophores. COL did not inhibit the *in vivo* photolabeling of Pgp by a vinblastine analog, whereas vinca alkaloids, verapamil and diltiazem were able to do so.[19,20] Both calcium channel blockers, verapamil and diltiazem, could inhibit Pgp photolabeling by a cyclosporin analog, whereas COL had no effect.[7] A series of tested RMA could restore DAU retention by MDR-P388 cells,[8] while several of them could not do so when another Pgp substrate, rhodamine-123, was used.[12]

Therefore Pgp-directed chemosensitizers that would interfere with the Pgp pump function at the level of the drug pharmacophore(s) might affect differentially the MDR cell resistance to distinct drugs. It could be expected that chemosensitizers that would cause more drastic inhibition of the Pgp molecules, such as inducing alterations of the ter-

tiary structure or quaternary organization in the plane of the membrane, would display little or no drug or Pgp substrate selectivity. In this study we compared the novel cyclopeptolide SDZ 280-125 with CsA, particularly because the *in vitro* RM activity of this new (non-IS) compound fell within the same range as that of CsA.

CsA has entered clinical trials for reversing multidrug-resistance worldwide,[20–23] but has the disadvantage of being a very potent IS agent. The new fungeal cyclic peptolide SDZ 90-215 was chemically characterized[16] as being Cyclo[Pec[1]-MeVal[2]-Val[3]-MeAsp[4]-Melle[5]-Melle[6]-Gly[7]-MeVal[8]-Tyr(Me)[9]-D-Lact[10]]. While the parent cyclopeptolide was completely devoid of RM activity, its derivative SDZ 280-125 could reverse MDR in cell lines expressing high levels of Pgp, its chemosensitizing activity being a few times higher than the one displayed by CsA.

Using the same pairs of cell lines as in the present study, CsA was compared with a variety of other RMA, among which verapamil, quinidine and amiodarone, and it was shown to be more potent in restoring *in vitro* the ACD sensitivity of MDR-CHO cells[5] and the DAU retention by MDR-P388.[8] Thus the higher strength of CsA as a RMA for Pgp-mediated MDR was shown by two very different assays, since the read-out for the cell growth inhibition assay was performed after several days of culture in the continuous presence of the RMA with CHO cells, whereas the DAU retention assay was performed after a 30 min pulse exposure of P388 cells to the RMA whose capacity to retain the anthracycline was then measured within the following hour. In spite of these very different experimental conditions, the relative levels of RM activity were preserved for all RMA tested so far and this was the case in the present study as well. This should validate our evaluation of a roughly 3-fold higher *in vitro* RM activity for SDZ 280-125 than for CsA.

For completely restoring ACD-mediated MDR-CHO cell growth inhibition, 2–5 μM concentrations of both SDZ 280-125 and CsA were required. Yet, at lower doses, in the linear parts of the dose–response curves, the comparison of their capacity to reverse Pgp-mediated MDR showed the higher strength of SDZ 280-125: thus, about 3-fold (range of 2.3–3.9 times, depending on the ACD) more CsA than cyclopeptolide SDZ 280-125 was required to give a 10-fold sensitization. Similarly, for the restoration of the capacity to accumulate and retain an antineoplastic anthracycline, SDZ 280-125 was about 3 times more active than CsA. Since, in the CHO cell growth inhibition assay, the direct cytotoxicity of SDZ 280-125 alone was similar to the one of CsA, the novel cyclopeptolide SDZ 280-125 allowed a wider *in vitro* therapeutic window than CsA, its RMA property being obtained at lower concentrations.

Since the profile of RM activity of SDZ 280-125 was like that of CsA, SDZ PSC 833 or SDZ 280-446, what could be the interest of using SDZ 280-125? In comparison with SDZ 280-446 and other L-Lact[10] derivatives of the original SDZ 90-215, the interest in SDZ 280-125 and other D-Lact[10]-cyclopeptolide would be in their easier chemical synthesis, since they do not need isomerization, thus their easier availability. In comparison with CsA, it would be their lack of IS activity. However, in comparison with stronger chemosensitizers, the easier availability of SDZ 280-125 might not compensate for a need for larger amounts to reverse the MDR character of tumor cells. Indeed, SDZ 280-125 remains an order of magnitude weaker than our previously described semi-synthetic cyclopeptolide derivative SDZ 280-446,[11] and the cyclosporin derivative SDZ PSC 833.[6,25]

Extensive knowledge on the pharmacology of cyclosporins has accumulated over the years from clinical experience in transplantation and autoimmunity. Since SDZ PSC 833 does not display significant IS activity and shows the advantage of being produced by a single step chemical modification of the naturally occurring cyclosporin (CsD), it should be the most suitable choice for experiencing clinical practice with this new cyclic peptide/peptolide class of chemosensitizers. However, development of a second generation of such RMA should be considered and offer potential, even though still largely hypothetical, advantages over SDZ PSC 833.

Although Pgp is a normal component of the plasma membrane of a variety of cells in the body,[26–30] its normal function on such normal cells is still unknown. One could speculate that the avidity of SDZ 280-125 for Pgp might be lower than the one displayed by SDZ PSC 833 and SDZ 280-446. Then the advantage of a compound with an intrinsically lower chemosensitizing activity such as SDZ 280-125 might be a more easily reversible RMA-dependent inhibition of Pgp function. If such is the case, it might be easier to modulate its inhibitory activity of Pgp by adjustments of the dosage and frequency of *in vivo* applications of the RMA, than with chemosensitizers displaying very high avidity for the Pgp. Treatment of experimental animals with very large amounts of our strongest RMAs SDZ PSC 833 and SDZ 280-446 has surprisingly failed to show toxic sequels, which may indicate that there is no real

requirement for the use of a soft chemosensitizer for preserving the normal function of normal Pgp-expressing cells.

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