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Short communication

Comparison of cyclosporin A and SDZ PSC833 as multidrug-resistance modulators in a daunorubicin-resistant Ehrlich ascites tumor

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Summary. Recent studies by Boesch et al. have demonstrated that a nonimmunosuppressive cyclosporin analog, SDZ PSC 833 (an analog of cyclosporin D), is an active multidrug-resistance modifier that is at least 10 times more potent than cyclosporin A. In vitro accumulation and cytotoxicity experiments using daunorubicin (DNR) and vincristine (VCR) under the influence of SDZ PSC 833 and cyclosporin A were performed in wild-type (EHR2) and the corresponding highly DNR-resistant (about 80-fold) Ehrlich ascites tumor cells (EHR2/DNR+). In accumulation experiments, both SDZ PSC 833 and cyclosporin A were found to reverse the multidrug-resistant (MDR) phenotype, but to the same degree at equimolar concentrations. Thus, in EHR2/DNR+ cells, both cyclosporins at 5 μg/ml enhanced DNR and VCR accumulation to sensitive levels, but only a negligible effect on DNR accumulation in the drug-sensitive cells was seen. In the clonogenic assay, the cytotoxicity of the two modulators was equal. The lethal dose for 50% of the cell population (LD_{50}) was approx. 7 μg/ml for both compounds, and no toxicity was observed at concentrations below 2 μg/ml. At nontoxic doses, both cyclosporins effectively increased the cytotoxicity of DNR and VCR in a concentration-dependent manner. The dose-response curves were nearly identical and did not demonstrate differences in modulator potency. These data permit the conclusion that cyclosporin A and SDZ PSC 833 do raise the intracellular accumulation of DNR and VCR to the same levels and that SDZ PSC 833 does not potentiate cytotoxicity better than cyclosporin A in EHR2/DNR+ cells. However, since the new compound is nonimmunosuppressive and causes less organ toxicity, clinical studies of its MDR modulating effect seem highly relevant.

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Introduction

One of the major obstacles to successful anticancer chemotherapy is the relatively rapid emergence of drug-resistant subpopulations of cells in tumors. These cells often display a multidrug-resistant (MDR) phenotype, i.e., they are cross-resistant to several chemically unrelated drugs (anthracyclines, vinca alkaloids, and epipodophyllotoxins [6, 15]) with different mechanisms of action.

Numerous reports have been published in recent years concerning the reversal of MDR by different modulators, including cyclosporin A (CyA) [3, 4, 21, 22]. These modulators are thought to act by inhibiting outward transport of the active drugs [12]. Slater et al. [20] have demonstrated that CyA reverses vincristine (VCR) resistance, and Osieka et al. [14] have shown that CyA potentiates the cytotoxicity of etoposide (VP-16) and doxorubicin by inhibiting drug efflux. These effects on MDR agents are probably caused by a competitive binding of CyA to P-glycoprotein (Pgp) as demonstrated by immunoprecipitation with the monoclonal antibody C219 [9].

SDZ PSC 833 is an analog of cyclosporin D that has been found in preliminary experiments to be less toxic than CyA and more effective in reversing MDR [5]. We have previously demonstrated that CyA is capable of modulating daunorubicin (DNR) [11] and VCR (data not shown) resistance by enhancing their accumulation to sensitive levels. In the present study we compared the influence of CyA and the new analog SDZ PSC 833 on DNR and VCR accumulation and cytotoxicity in DNR-resistant Ehrlich ascites tumor cells.

Materials and methods

Chemicals. DNR hydrochloride was obtained from Farmitalia, Carlo Erba (Milan, Italy) and was dissolved in water as a 1-mM stock solution. [^{3}H]-VCR (7.2 Ci/mmol) was purchased from Amersham International (UK). CyA and SDZ PSC 833 (powders) were supplied by Sandoz Pharma AG (Basel, Switzerland). CyA and the analog were dissolved in ethanol and diluted in the samples to a final ethanol concentration of 1%. All other chemicals were of analytical grade.

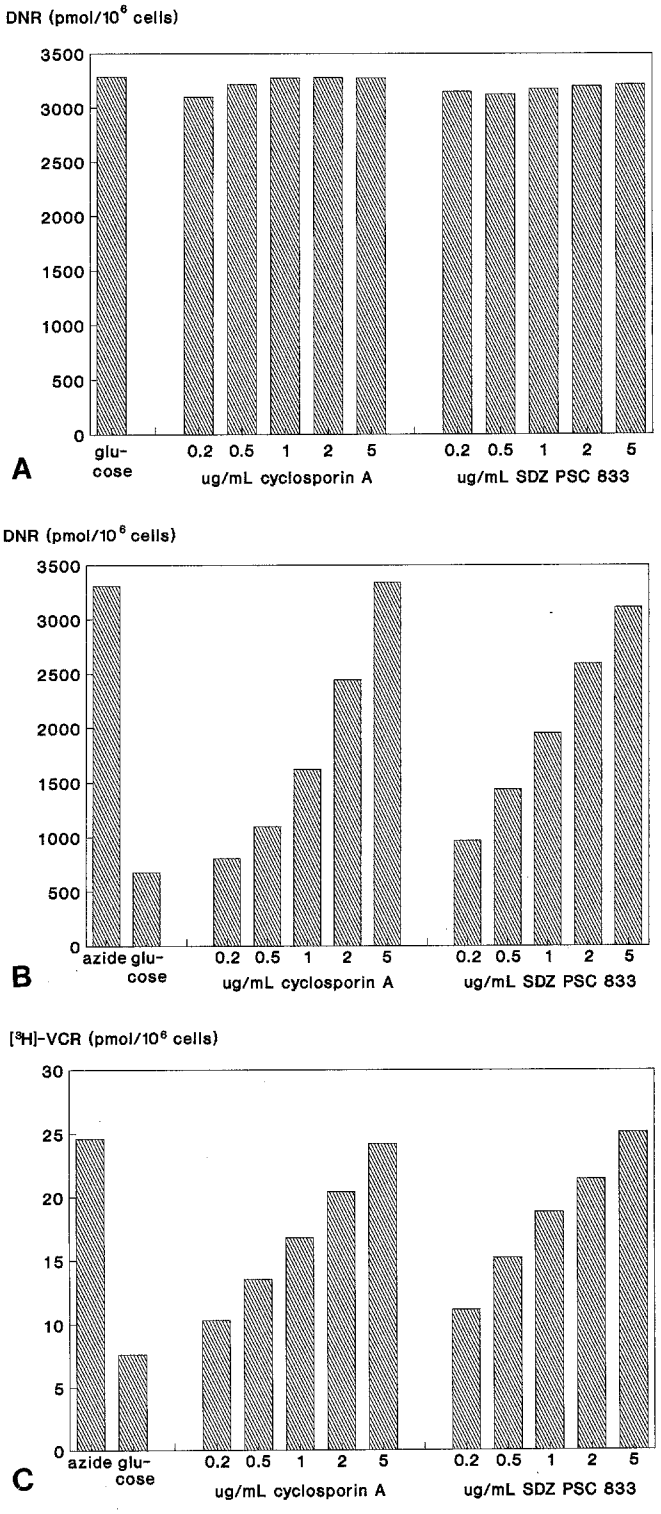

Fig. 1 A–C. Effects of CyA and SDZ PSC 833 on the steady-state accumulation of **A** DNR in drug-sensitive cells and **B** DNR or **C** [^{3}H]-VCR in drug-resistant cells. Suspensions of 10^6 cells/ml were incubated for 60 min in standard medium containing 10 mM glucose at 37° C with 5 μM DNR or 1 μM [^{3}H]-VCR and different concentrations of the two modulators as indicated. Each bar represents the mean of 3 separate determinations; the SD constitutes ±1% of the mean values

Cell lines. The cell lines used included the Ehrlich ascites tumor line (EHR2) and the corresponding DNR-resistant line (EHR2/DNR+) [7], which displays all of the characteristics of the classic MDR phenotype [8, 10, 18, 19]. These two cell lines were developed [7] and maintained in vivo as described elsewhere [7, 11]. The cells were counted in a Bürker-Türk hemocytometer, and the final cell suspension was adjusted to 10^6 cells/ml.

Drug accumulation. Cells were incubated for 60 min at 37° C in phosphate buffer [11] supplemented with 10 mM glucose (pH 7.45). Dialyzed 5% (v/v) calf serum was added to the medium in all trials. After 60 min, the experiments were terminated by transferring 2.0 ml cell suspension into ice-cold Ringer's solution. The cells were pelleted at 3000 *g* for 5 min and washed twice with 7 ml ice-cold Ringer's solution. The cellular accumulation of DNR under the influence of CyA or SDZ PSC 833 was determined by the extraction of DNR from the cells with a solution of 0.3 *N* HCl/50% ethanol followed by spectrofluorometry (excitation wavelength, 470 nm; emission wavelength, 585 nm) [1]. The cellular content of [^{3}H]-VCR was determined as previously described [11].

Clonogenic assay. Drug toxicity was assessed by colony formation in soft agar on a feeder layer containing sheep red blood cells as described elsewhere [13, 17]. Continuous drug incubation was used.

Results and discussion

Reduced intracellular drug accumulation, expression of Pgp [2, 16], and reversibility by several classes of membrane-active agents [3] that increase the intracellular drug accumulation characterize the MDR phenotype. Figure 1 A and Fig. 1 B show the effects we observed on the intracellular accumulation of DNR in EHR2 and Pgp-positive EHR2/DNR+ cells after 1 h incubation with 5 μM DNR together with increasing concentrations of CyA and SDZ PSC 833. At identical concentrations, CyA and SDZ PSC 833 seemed to increase steady-state DNR accumulation to nearly the same level. The maximal enhancement in accumulation in DNR-resistant cells was obtained using 5-μg/ml concentrations of both cyclosporin analogs and corresponded to the level of DNR measured in the sensitive cells. Neither of the agents modulated DNR accumulation in the sensitive cells.

To determine whether CyA and SDZ PSC 833 would also enhance the accumulation of a chemically different drug exhibiting the MDR phenotype to the same degree, we examined their effects on the accumulation of [^{3}H]-VCR. Figure 1 C shows that the two modulators also enhanced [^{3}H]-VCR accumulation to sensitive levels. We found the effect of the two cyclosporins to be concentration-dependent. At concentrations exceeding 5 μg/ml, no further increase in the accumulation of DNR or VCR was seen, although a toxic effect in agreement with previous findings was noted [11].

Using a clonogenic assay, we examined the in vitro cytotoxicity of DNR and VCR combined with either CyA or SDZ PSC 833 in EHR2/DNR+ cells. The results are shown in Fig. 2. CyA and SDZ PSC 833 caused little in vitro toxicity in EHR2/DNR+ cells, with the LD_{50} values being approx. 7 μg/ml. Both CyA and SDZ PSC 833 modulated DNR and VCR resistance, but in contrast to Boesch et al. [5], who found SDZ PSC 833 to be at least 10 times more active than CyA, we found no significant

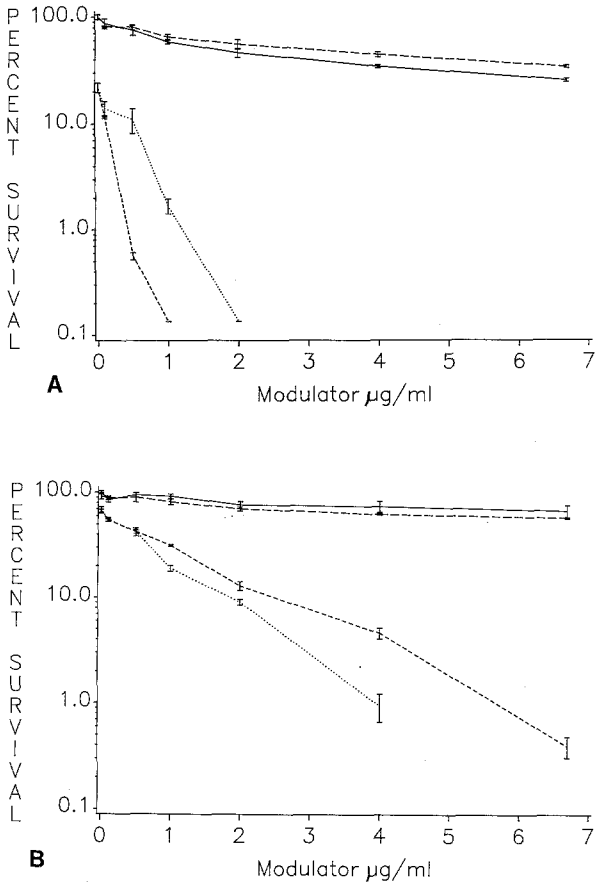

Fig. 2 A, B. In vitro cytotoxicity of CyA or SDZ PSC 833 alone or in combination with **A** 1 μM DNR or **B** 0.2 μM VCR in EHR2/DNR+ cells. Colonies were counted after continuous exposure of cells to the drugs for 3 weeks as described elsewhere [17]. Bars represent standard errors from triplicate cultures. **A** ———, CyA; – –, SDZ PSC 833; – – – –, DNR+CyA; . . . . , DNR + SDZ PSC 833. **B** – – – – VCR+CyA; . . . . . , VCR, + SDZ PSC 833

difference in the modulating effect of the cyclosporins on DNR or VCR cytotoxicity. Thus, the dose-response curves were nearly identical and did not demonstrate differences in modulator potency. Using three analogs of CyA, Twentyman et al. [22] found a close correlation between the immunosuppressive efficiency and the chemosensitizing capacity of CyA analogs in vitro. This tight correlation was not observed in the study by Foxwell et al. [9], and our data also indicate that the immunosuppressive effect is not an imperative element.

In conclusion, our study gave no indication that SDZ PSC 833 was a more potent MDR modifier than CyA, but as the former is a non-immunosuppressive drug and therefore does not induce the side effects caused by the latter, the use of SDZ PSC 833 as an MDR modifier would be preferable in future clinical studies. Furthermore, the testing of additional CyA analogs may disclose the existence of noncytotoxic agents that exhibit only low, if any, immunosuppressive activity and an even more potent modulating capacity.

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References

1. Bachur NR, Moore AL, Bernstein JG, Liu A (1970) Tissue distribution and disposition of daunomycin (NSC-82 151) in mice: fluorometric and isotopic methods. Cancer Chemother Rep 54: 89–94
2. Beck WT (1987) The cell biology of multiple drug resistance. Biochem Pharmacol 36: 2879
3. Beck WT (1991) Modulators of P-glycoprotein-associated multidrug resistance: In: Molecular and clinical advances in anticancer drug resistance. Kluwer, Boston, p 151
4. Boesch D, Gavériaux C, Loor F (1991) Reversal of multidrug resistance in CHO cells by cyclosporin A and other resistance modifying agents. J Cell Pharmacol 2: 92
5. Boesch D, Gavériaux C, Jachez B, Pourtier-Manzanedo A, Bollinger P, Loor F (1991) In vitro circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. Cancer Res 51: 4226
6. Bradley G, Juranka PF, Ling V (1988) Mechanism of multidrug resistance. Biochim Biophys Acta 948: 87
7. Danø K (1971) Development of resistance to daunomycin (NSC-82 151) in Ehrlich ascites tumor. Cancer Chemother Rep 55: 133
8. Danø K (1973) Active outward transport of daunomycin in resistant Ehrlich ascites tumor cells. Biochim Biophys Acta 323: 466
9. Foxwell BMJ, Mackie A, Ling V, Ryffel B (1989) Identification of the multi-drug resistance related P-glycoprotein as a cyclosporine binding protein. Mol Pharmacol 36: 543
10. Friche E, Skovsgaard T, Nissen NI (1987) Effect of verapamil on daunorubicin accumulation in Ehrlich ascites tumor cells. Cancer Chemother Pharmacol 19: 35
11. Friche E, Jensen PB, Sehested M, Demant EJF, Nissen NI (1990) The solvents Cremophor EL and Tween 80 modulate daunorubicin resistance in the multidrug resistant Ehrlich ascites tumor. Cancer Commun 2: 297
12. Georges E, Sharom FJ, Ling V (1990) Multidrug resistance and chemosensitization: therapeutic implications for cancer chemotherapy. Adv Pharmacol 21: 185
13. Jensen PB, Roed H, Vindeløv LL, Christensen IJ, Hansen HH (1989) Reduced variation in the clonogenic assay obtained by standardization of the cell culture conditions prior to drug testing on human small cell lung cancer cell lines. Invest New Drugs 7: 307
14. Osieka R, Seeber S, Pannenbiacker R, Sall D, Glatte P, Schmidt CG (1986) Enhancement of etoposide-induced cytotoxicity by cyclosporin A. Cancer Chemother Pharmacol 18: 298
15. Pastan I, Gottesman MM (1987) Multidrug resistance in human cancer. N Engl J Med 316: 1388
16. Riordan JR, Ling V (1985) Genetic and biochemical characterization of multidrug resistance. Pharmacol Ther 28: 51
17. Roed H, Christensen IJ, Vindeløv LL, Spang-Thomsen M, Hansen HH (1987) Inter-experiment variation and dependence on culture conditions in assaying the chemosensitivity of human small cell lung cancer lines. Eur J Cancer Clin Oncol 23: 177
18. Sehested M, Jensen PB, Skovsgaard T, Bindslev N, Demant EJF, Friche E, Vindeløv L (1989) Inhibition of vincristine binding to plasma membrane vesicles from daunorubicin-resistant Ehrlich ascites cells by multidrug resistance modulators. Br J Cancer 60: 809
19. Skovsgaard T (1978) Mechanism of cross-resistance between vincristine and daunorubicin in Ehrlich ascites tumor cells. Cancer Res 38: 4722
20. Slater LM, Sweet P, Stupecky M, Gupta S (1986) Cyclosporin A reverses vincristine and daunorubicin resistance in acute lymphatic leukemia in vitro. J Clin Invest 77: 1405
21. Twentyman PR (1988) A possible role for cyclosporins in cancer chemotherapy. Anticancer Res 8: 985
22. Twentyman PR, Fox NE, White DJG (1987) Cyclosporin A and its analogues as modifiers of Adriamycin and vincristine resistance in a multi-drug resistance human lung cancer cell line. Br J Cancer 56: 55