OVERCOMING PGP-RELATED MULTIDRUG RESISTANCE. THE CYCLOSPORINE DERIVATIVE SDZ PSC 833 CAN ABOLISH THE RESISTANCE TO METHOXY-MORPHOLYNIL-DOXORUBICIN

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ABSTRACT

Background. The results obtained so far in studies designed to neutralize P glycoprotein (PGP)-related multidrug resistance (MDR) by using MDR reversal agents, have not yet fulfilled the promise of the experiments which were performed *in vitro*. In order to improve PGP-related MDR neutralization, we tested *in vitro* the activity of the cyclosporine derivative SDZ PSC 833 (PSC) together with doxorubicin (DOX) and with two new DOX derivatives named 4' iodo 4' deoxy-doxorubicin (IODODOX) and methoxy-morpholynil-doxorubicin (MMDOX, FCE 23762) using four different human cell lines and their multi-drug resistant variants.

Methods. Anthracycline toxicity was evaluated by using the MTT method after a 7-day culture with continuous exposure to the antitumor drugs with or without the addition of PSC.

Results. PSC significantly downmodulated the toxicity of all three anthracyclines in all the four cell systems. However, despite the great increase caused by PSC in the toxicity of DOX and a more modest effect on the toxicity of the two DOX derivatives, this MDR reversal agent could only completely block the PGP mediated MMDOX resistance whereas DOX refractoriness was only decreased.

Conclusions. The combination of MMDOX or IODODOX with PSC 1.6 µM is more efficient than the combination of DOX plus PSC for the full reversion of PGP-mediated drug resistance. Careful clinical studies are required to evaluate if these associations can also effectively and safely neutralize MDR *in vivo*.

Keywords: multidrug resistance, P-glycoprotein, reversal agents, anthracyclines

A number of experimental studies showed that the multi drug resistance (MDR) caused by the overexpression of the P-glycoprotein (PGP), can be successfully blocked *in vitro* by several different methods including the use of a wide range of drugs named MDR reversal agents.[1-5] However, the results of the pilot clinical trials designed to overcome MDR *in vivo* did not fulfill the promise of previous *in vitro* observations.[6-11] While this could reflect a limited clinical relevance of the MDR phenomenon, the scarce results obtained in these clinical trials could also be explained by the use of inappropriate MDR reversal agents or by an inappropriate association with antitumor drugs. In fact, in these first studies drugs as verapamil, quinidine or cyclosporine A were used as MDR reversal agents. It should be noticed that these drugs were not originally designed for the neutralization of the MDR phenomenon. Thus, in the majority of the studies the doses of these modulators were below those required to achieve effective inhibition of PGP function *in vitro*, because of their dose-limiting toxicity. Moreover, all the studies that were performed up to now tried to downmodulate the resistance to drugs that, like doxorubicin, daunorubicin, etoposide or vincristine, are quickly captured and efficiently pumped out by the PGP.[6-16] In the past 20 years, many efforts

were devoted to select new reversal agents lacking the dose limiting toxicities and to synthesize new antitumor derivatives that are not transported by the P-glycoprotein.[17-27] With the aim of optimizing the neutralization of PGP-MDR through the use of reversal agents, we tested two lyphofilic doxorubicin (DOX) derivatives that are more toxic than the parent compound against MDR cells, namely 4'iodo 4'deoxy DOX (IODODOX) and methoxy-morpholynil DOX (MMDOX) together with the cyclosporine derivative SDZ PSC 833 (PSC). This is one of the most efficient MDR reversal agents developed and tested so far.[28-33] In comparison with DOX, it was found that the combination of both compounds with PSC was highly efficient and could abolish PGP-related resistance.

Materials and Methods

Drugs

Doxorubicin (DOX) was purchased from Pharmacia S.p.A. Milano, Italy. The two DOX-derivatives 4'iodo4'deoxyDOX (IODODOX) and methoxy-morpholynil-DOX (MMDOX, FCE 23762) were a gift from Pharmacia S.p.A. SDZ PSC 833 was a gift from Sandoz, Basel. DOX and IODODOX were dissolved in water at 100 µg/mL. MMDOX and PSC were dissolved in ethanol at 1 and 5 mg/mL, respectively. All the drugs were stored at –20° and were immediately thawed and diluted before use.

Cell lines

Four systems of human cell lines were used. Each system included a parental, drug sensitive, line without the amplification of the mdr-1 gene and PGP overexpression, and one drug-selected MDR variant with mdr-1 gene amplification and PGP overexpression. The first cell system included the acute myeloid leukemia HL60 cell line and its daunorubicin-selected variant HL60DNR.[34] The second system included the acute lymphocytic leukemia cell line CCRFCEM and its vinblastine-selected variant CEMVLB300.[23] The third system included the colon adenocarcinoma cell line LOVO109 and its DOX-selected variant LOVODOX[23] The fourth system included the

breast cancer cell line MCF7 and its DOX-selected variant MCF7DOX.[34] All cell lines were cultured at 37°C in a humidified atmosphere of 5% CO_2 and maintained in an exponential growth in RPMI 1640 (Biochem KG Seromed) with 10% heat-inactivated foetal calf serum (Biochem KG Seromed), 2 mM glutamine solution, 100 U/mL penicillin and 100 µg/mL streptomycin (Biochem KG Seromed). A selected pressure of the appropriate antiblastic drug (HL60DNR, daunorubicin 0.4 µg/mL; CEMVLB300, vinblastine 0.3 µg/mL; LOVODOX, DOX 0.2 µg/mL; MCF7DOX, DOX 0.2 µg/mL) was constantly maintained just for the MDR cell lines. Before each drug sensitivity assay the PGP expression and the expression of other MDR-associated proteins (*lung resistance protein* or LRP, *multidrug resistance-associated protein* or MRP) and of the GSTπ enzyme were evaluated by a flow cytometry assay by using the MRK-16 (Kamiya), the LRP56 (Kamiya), the MRPm6 (Kamiya) and the GSTπ (Dako) monoclonal antibodies. PGP was evaluated as described further on.[35,36] The LRP56, the MRPm6 and the GSTπ monoclonal antibodies were used following the company guidelines. To quantitate the expression of these MDR associated proteins, the number of the MoAbs bound sites per cell was evaluated by using the Quantum Simply Cellular Kit (Sigma). The results were expressed in units of *antibody binding capacity* (ABC) as required for this method. Before the experiments the non MDR parental cell lines had an ABC of 177×10^3 (HL60), 98×10^3 (CCRFCEM), 65×10^3 (LOVO 109) and 38×10^3 (MCF7). The respective MDR cell lines had an ABC of 3650×10^3 (HL60DNR), 1477×10^3 (CEMVLB300), 6903×10^3 (LOVODOX), and 7517×10^3 (MCF7DOX). No drug selected cell line variants overexpressed LRP, MRP or GSTπ in comparison with the respective parental sensitive cell lines, except LOVODOX, whose reactivity to LRP56 was about three times more than in LOVO109.

Drug sensitivity assay

Cell growth in presence or absence of drugs was determined by using the MTT-microcultured tetrazolium colorimetric assay, as described elsewhere.[24,25] Briefly, exponential

growing cells were harvested, washed twice in RPMI 1640 (Biochrom KG Seromed), checked for their vitality through the tripan blue exclusion test, and plated into 96 well microtiter plates at the required concentration in 200 μL of a complete culture medium (RPMI 1640 plus 10% fetal calf serum, 2 mM glutamine solution, penicillin and streptomicin). After a 24-hour incubation, ever increasing doses of anthracyclines (0.5-3000 ng/mL) with or without PSC 1.6 μM were added. Cell growth was evaluated after a 7-day incubation at 37°C and 5% CO_2, by using 50 μL per well of the MTT solution (5 mg/mL). Formazan crystals were dissolved in DMSO and their optical density (OD) was read at 540 nm. As required, wells containing no drugs or containing PSC were used to control cell viability. Wells containing no cells were used to blank the spectrophotometer (Novapath Microplate Reader, BioRad). The inhibition dose (ID) was calculated according to Pieters *et al.*[37] by the following equation, where ID=(mean OD treated wells/mean OD control wells) ×100. Every point of the dose-response curves was the mean of three tests at least. The ID_{50} was defined as the drug dose that inhibited the cell growth to 50% of the control. The resistance index (RI) was calculated by dividing the ID_{50} of the MDR cell line with the ID_{50} of the respective non MDR cell line.

Results

For all the MDR and non MDR cell lines the dose response curves obtained by testing the toxicity of all the anthracyclines in presence or in absence of PSC, were drawn to calculate the inhibition dose 50 (ID_{50}) and the resistance index (RI). Table 1 summarizes all the ID_{50} obtained testing DOX. As expected DOX was several times less toxic in the MDR cell lines than in the parental ones. In the parental cell lines PSC was inactive, whereas in resistant cells PSC the resistance to DOX was substantially decreased. In fact, in the MDR cell lines the ID50 fell from 433 to 27 ng/mL (CEMVLB300), from 1477 to 30 ng/mL (HL60DNR), from 718 to 15 ng/mL (LOVODOX) and from 1230 to 48 ng/mL (MCF7DOX) (Table 1). However, despite the impressive reductions of the ID_{50} caused by PSC, in the MDR cell lines the sensitivity to DOX never reached the level of the respective parental lines. A possible exception was LOVODOX. Table 2 reports the toxicity of IODODOX alone or in presence of PSC in the MDR and non MDR cell lines. In the MDR cell lines, IODODOX was by itself more toxic than the parental agent DOX. In fact, as shown in Table 2, the RI was 9.4 for the CEM system (CEMVLB300/CCRFCEM), 17.7 for the HL60 system (HL60DNR/HL60), 4 for the LOVO system (LOVODOX/LOVO109) and 3 for the MCF7 system (MCF7DOX/MCF7). Once again the addition of PSC increased the anthracycline toxicity only in the MDR cell lines. Thus, the ID_{50}s of IODODOX fell from 18 to 7.7 ng/mL in CEMVLB300, from 55 to 6.8 ng/mL in HL60DNR, from 26 to 9.9 in LOVODOX and from 57 to 26 ng/mL in MCF7DOX. Therefore, for IODODOX an almost complete neutraliza-

MDR/non MDR cell lines	Doxorubicin		Doxorubicin Plus PSC 1.6 μM	
	Inhibition Dose 50 (ng/mL)	Resistance Index	Inhibition Dose 50 (ng/mL)	Resistance Index
CEM VLB300/CCRF CEM	433/5.5	79	27.0/5.4	5.0
HL60 DNR/HL60	1477/7.6	194	30.0/7.2	4.2
LOVO DOX/LOVO109	718/8.6	83	15.0/8.0	1.8
MCF7 DOX/MCF7	1230/10	123	48.0/9.2	5.2

Table 1. Inhibition dose$_{50}$ (ID_{50}) and resistance index (RI) obtained testing doxorubicin with or without PSC 1.6 μM in the MDR and non MDR cell lines. In all the MDR cell lines PSC significantly increased doxorubicin toxicity. However this MDR reversal agent could not neutralize completely the resistance to doxorubicin, with a possible exception for the LOVODOX/LOVO109 system, where the RI became close to 1.

MDR/non MDR cell lines	Iodo-doxorubicin		Iodo-doxorubicin Plus PSC 1.6 μM	
	Inhibition Dose 50 (ng/mL)	Resistance Index	Inhibition Dose 50 (ng/mL)	Resistance Index
CEM VLB300/CCRF CEM	18.0/1.9	9.4	7.7/1.3	5.9
HL60 DNR/HL60	55.0/3.1	17.7	6.8/2.8	2.4
LOVO DOX/LOVO109	26.0/6.5	4.0	9.9/6.2	1.6
MCF7 DOX/MCF7	57.0/19	3.0	26.0/19.0	1.4

Table 2. Inhibition dose$_{50}$ (ID$_{50}$) and resistance index (RI) obtained testing iodo-doxorubicin with or without the addition of PSC 1.6 μM in the MDR and non MDR cell lines. The addition of PSC decreased all the ID$_{50}$ in all the MDR cell lines. A RI closed to one was obtained in LOVODOX/LOVO109 and MCF7DOX/MCF7 systems.

MDR/non MDR cell lines	Methoxy-morpholynil doxorubicin		Methoxy-morpholynil doxorubicin plus PSC 1.6 μM	
	Inhibition Dose 50 (ng/mL)	Resistance Index	Inhibition Dose 50 (ng/mL)	Resistance Index
CEM VLB300/CCRF CEM	7.8/4.4	1.7	2.8/4.4	0.6
HL60 DNR/HL60	27.2/3.4	8.0	6.8/3.6	1.9
LOVO DOX/LOVO109	21.6/6.8	3.1	8.4/6.4	1.3
MCF7 DOX/MCF7	17.0/13.0	1.3	15.3/13.3	1.2

Table 3. Inhibition dose$_{50}$ (ID$_{50}$) and resistance index (RI) obtained testing methoxy-morpholynil doxorubicin in presence or in absence of PSC 1.6 μM in the MDR and non MDR cell lines. MMDoxorubicin was very toxic against MDR cells also when it was used alone. When it was used together with PSC, the RI become close to 1 in all four cell systems, showing that this combination had the potential of abolishing PGP-related resistance.

tion of PGP activity, that means a RI close to 1, was obtained in LOVODOX/LOVO109 (RI = 1.6) and in MCF7DOX/MCF7 (RI = 1.4) cell lines. Table 3 summarizes the results obtained with MMDOX. In the drug resistant cell lines MMDOX itself was highly toxic. Its power was particularly evident in MCF7DOX and in CEMVLB300 where the dose response curves of MMDOX alone were very close to the curves obtained in the respective parental cell lines (Figure 1). The coincubation of this anthracycline derivative with PSC further increased MMDOX toxicity in the MDR cell lines so that the addition of PSC could almost completely neutralize the residual resistance to MMDOX (Table 3 and Figure 1).

Discussion

SDZ PSC 833 (PSC) is a novel non immunosuppressive analog of cyclosporine. It was recently completed phase-1 trials which show that at the maximum tolerated dose plasma levels ranged between 2 and 4 μg/mL (1.6-3.2 μM).[19,28,29] Laboratory studies showed that at these concentration ranges PSC can provide highly efficient chemosensitization, but also that a complete MDR elimination may be compromised by a high overexpression of the mdr-1 gene.[19,24,30-33] The new anthracyclines IODODOX and MMDOX where shown to be very powerful DOX derivatives.[38-41] Compared to their drug parent, they are characterized by a higher lipophilicity which seems to be the basis of their rapid spreading through the cell membrane and also by their higher intracellular accumulation in MDR cells.[39,40,42,43] With the aim of optimizing the neutralization of PGP-related MDR through the use of reversal agents we selected the two best available DOX derivatives (IODODOX and MMDOX). We tested their toxicity in comparison with DOX, with or without the addition of PSC in an experimental model of four different human cell line systems, including a sensitive line and a PGP-overexpressing MDR variant. Our findings confirmed prior reports, showing that both DOX derivatives alone were more toxic than DOX in the MDR cell lines.[25,26,42-48] However,
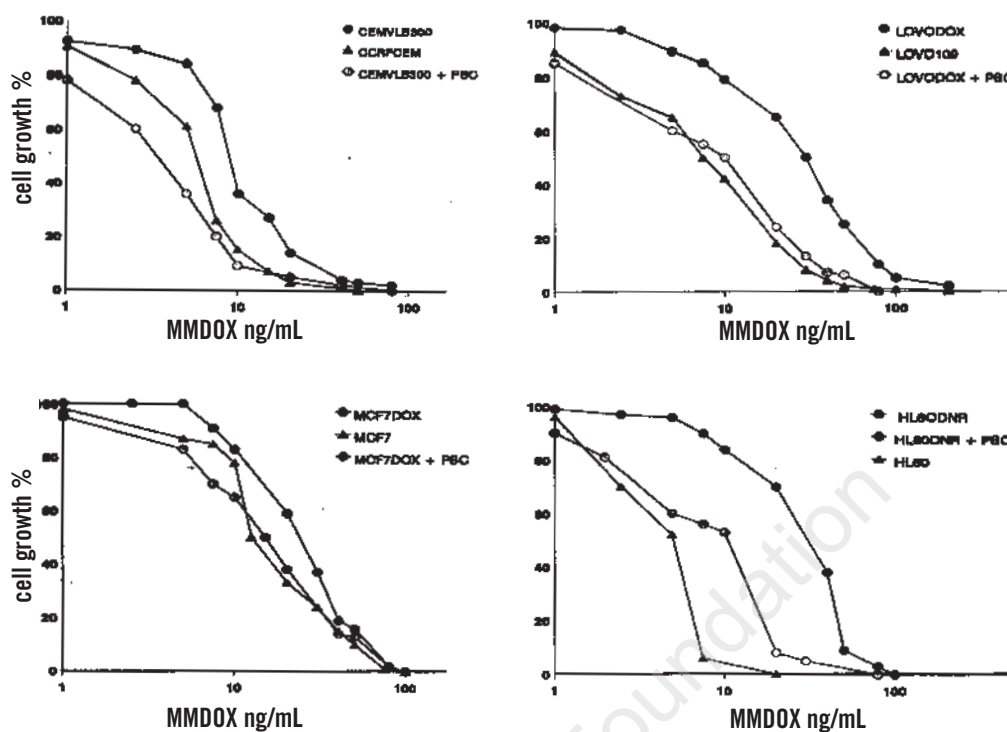
Figure 1. Dose response curves of methoxy-morpholynil doxoruvicin (MMDOX) with (open symbols) or without (closed symbols) SDZ PSC 833 (PSC) 1.6 µM in the four cell systems which were composed of parental, drug sensitive, line (CCRFCEM, LOVO109, MCF7, HL60) and its drug-selected MDR variant (CEMVLB300, LOVODOX, MCF7DOX, HL60DNR). In the drug-resistant cell lines (closed circles), MMDOX was less toxic than in the parental cell lines (closed triangles). In all the four MDR-resistant cell lines the addition of MMDOX increased the toxicity of MMDOX and almost completely neutralized PGP-related resistance.

to completely remove the anthracycline resistance caused by PGP in an experimental model based on MDR positive and negative cells, it is necessary that in the presence of the reversal agent, the ID_{50} of the MDR positive cell line variant must equal the ID_{50} of the parental line. In other words, the RI must fall close to 1. In our example, PSC at 1.6 µM which was shown to be a safely achievable concentration also *in vivo*, was inactive in the non MDR cell lines, while it influenced the toxicity of all the anthracyclines in the MDR cell lines. The power of PSC in blocking PGP was particularly evident when it was used in combination with DOX. In fact this association could reduce DOX ID_{50} more than 15 fold, while the ID_{50} of IODODOX and MMDOX was only reduced by 2-5 folds. However, despite the great effect in sensitizing DOX and the apparently modest effect in sensitizing DOX derivatives, when the toxicity of the anthracyclines plus PSC in the MDR cell lines

was compared with the toxicity in the non MDR cell lines, it emerged that only MMDOX resistance could be almost completely abolished. On the contrary, resistance to DOX only decreased.

A possible exception was LOVODOX; in this cell line, PSC was highly efficient in blocking PGP, and in the case of DOX it could reduce the RI to almost 1. The discrepant effect of reversal agents in counteracting the drug resistance to different drugs in different cell lines has already been described.[49] A possible explanation could be that cell lines selected with a positive drug pressure can simultaneously develop different mechanisms of resistance. In our cell lines, an amplification of the mdr-1 gene has already been described and a high PGP overexpression was confirmed in our laboratory by using the MRK-16 MoAb and functional assays.[23,33] To better define the mechanisms of MDR in our cell lines, other possible MDR factors were investigated. This screening included the evaluation of the

expression of two other drug transporter proteins which belong to the ATP binding cassette superfamily, called the *lung resistance protein* (LRP)[50] and the *multidrug resistance-associated protein* (MRP),[51] as well as the study of the expression of the enzyme GSTπ.[52] We found that only LOVODOX displayed a modest increase of the LRP. Therefore, the results that were obtained in this study can apply specifically only to PGP-related MDR. Our observations show that PGP-related MDR can be almost completely removed by using PSC, even when tested cells have a very high PGP expression, if the parent drug DOX is substituted by its more lipophilic derivatives. This suggests that PSC is more efficient when it is used in association with drugs which by themselves can partially avoid the P-glycoprotein transport. Concerning the possible clinical application of these data, preliminary studies have already described the toxic, metabolic and pharmacokinetic properties of IODODOX, MMDOX and PSC respectively.[28,29,53-56] However, it must be pointed out that *in vitro* studies can describe and clarify the antitumor effect of a single drug or of a combination of drugs, but they can not predict therapeutic results and *in vivo* side-effects. Moreover, from the preclinical studies with reversal agents, including PSC, performed until now, we have learnt that the introduction of agents blocking the PGP-mediated drug efflux can also alter the antitumor drug metabolism, its delivery to the tumor and the pharmacokinetic.[12,16,29]

We conclude that the association of PSC and lipophilic anthracyclines like IODODOX and MMDOX is worth evaluating for antitumor effect and for toxicity in experimental models that is in high risk, primary chemoresistant human tumors.

References

1. Kaye SB. Reversal of multidrug resistance. Cancer Treat Rev 1990; 17:37-43.
2. Ramu A, Ramu N. Reversal of multidrug resistance by phenothiazines and structurally related compounds. Cancer Chemother Pharmacol 1992; 30:165-73.
3. Cano-Gauci DF, Riordan JR. Action of calcium antagonists on multidrug resistant cells. Biochem Pharmacol 1987; 36: 2115-23.
4. Merlin JL, Guerci A, Marchal S, et al. Comparative evaluation of S9788, verapamil, and cyclosporine A in K562 human leukemia cell lines and in P-glycoprotein-expressing samples from patients with hematologic malignancies. Blood 1994; 84:262-9.
5. Friche E, Skovsgaard T, Danø K. Multidrug resistance: drug extrusion and its counteraction by chemosensitizers. Eur J Haematol 1989; 42 (suppl. 48):59-67.
6. Raderer M, Scheithauer W. Clinical trials of agents that reverse multidrug resistance. Cancer 1993; 72:3553-63.
7. Gottesman MM, Pastan I. Clinical trials of agents that reverse multidrug-resistance. J Clin Oncol 1989; 7:409-11.
8. Yahanda AM, Adler KM, Fisher GA, et al. Phase I trial of etoposide with cyclosporine as a modulator of multidrug resistance. J Clin Oncol 1992; 10:1624-34.
9. Berman E, McBride M, Lin S, Menedez-Botet C, Tong W. Phase I trial of high-dose tamoxifen as a modulator of drug resistance in combination with daunorubicin in patients with relapsed or refractory acute leukemia. Leukemia 1995; 9: 1631-7.
10. Trump DL, Smith DC, Ellis PG, et al. High-dose oral tamoxifen, a potential multidrug-resistance-reversal agent: phase I trial in combination with vinblastine. J Natl Cancer Inst 1992; 84:1811-6.
11. Figueredo A, Arnold A, Goodyear M, et al. Addition of verapamil and tamoxifen to the initial chemotherapy of small cell lung cancer. Cancer 1990; 65:1895-902.
12. Sonneveld P, Durie BGM, Lokhurst HM, et al. Modulation of multidrug-resistant multiple myeloma by cyclosporin. Lancet 1992; 340:255-9.
13. Cairo MS, Siegel S, Anas N, Sender L. Clinical trial of continuous infusion verapamil, bolus vinblastine, and continuous infusion VP-16 in drug-resistant pediatric tumors. Cancer Res 1989; 49:1063-6.
14. Ozols RF, Cunnion RE, Klecker RW, et al. Verapamil and adriamycin in the treatment of drug-resistant ovarian cancer patients. J Clin Oncol 1987; 5:641-7.
15. Dalton WS, Grogan TM, Meltzer PS, et al. Drug-resistance in multiple myeloma and non-Hodgkin's lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. J Clin Oncol 1989; 7:415-24.
16. List AF, Spier C, Greer J, et al. Phase I/II trial of cyclosporine as a chemotherapy-resistance modifier in acute leukemia. J Clin Oncol 1993; 11:1652-60.
17. Jachez B, Boesch D, Grassberger MA, Loor F. Reversion of the P-glycoprotein-mediated multidrug resistance of cancer cells by FK-506 derivatives. Anti Cancer Drugs 1993; 4:223-9.
18. Twentyman PR. Modification of cytotoxic drug resistance by non-immuno-suppressive cyclosporins. Br J Cancer 1988; 57: 254-8.
19. Gaveriaux C, Boesch D, Jachez B, Bollinger P, Payne T, Loor F. SDZ PSC 833, a non-immunosuppressive cyclosporin analog, is a very potent multidrug-resistance modifier. J Cell Pharmacol 1991; 2:225-34.
20. Shudo N, Mizoguchi T, Kiyosue T, et al. Two pyridine analogoues with more effective ability to reverse multidrug resistance and with lower calcium channel blocking activity than their dihydropyridine counterparts. Cancer Res 1990; 20: 3055-61.
21. Hyafil F, Vergely C, Du Vignaud P, Grand-Perret T. *In vitro* and in vivo reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative. Cancer Res 1993; 53:4595-602.
22. Eliason JF, Ramuz H, Kaufmann F. Human multi-drug-resistant cancer cells exhibit a high degree of selectivity for stereoisomers of verapamil and quinidine. Int J Cancer 1990; 46:113-7.
23. Damiani D, Michieli M, Michelutti A, Melli C, Cerno M, Baccarani M. D-verapamil downmodulated P170-associated

resistance to doxorubicin, daunorubicin and idarubicin. Anti-Cancer Drugs 1993; 4:173-80.

24. Michieli M, Damiani D, Michelutti A, Melli C, Russo D, Fanin R, Baccarani M. P170-dependent multidrug resistance. Restoring full sensitivity to idarubicin with verapamil and cyclsporin A derivatives. Haematologica 1994; 79:119-26.

25. Michieli M, Michelutti A, Damiani D, et al. A comparative analysis of the sensitivity of multidrug resistant (MDR) and non-MDR cells to different anthracycline derivatives. Leuk Lymphoma 1993; 9:255-64.

26. Grandi M, Pezzoni G, Ballinari G, et al. Novel anthracycline analogs. Cancer Treat Rev 1990; 17:133-8.

27. Twentyman PR, Fox NE, Wright KA, et al. The in vitro effects and cross-resistance patterns of some novel anthracyclines. Br J Cancer 1986; 53:585-94.

28. Boesch D, Gaveriaux C, Jachez B, Pourtier-Manzanedo A, Bollinger P, Loor F. In vivo circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. Cancer Res 1991; 51:4226-33.

29. Keller RP, Altermatt HJ, Donatsch P, Zihlmann H, Laissue JA, Hiestand PC. Pharmacologic interactions between the resistance-modifying cyclosporine SDZ PSC 833 and etoposide (VP16-213) enhance in vivo cytostatic activity and toxicity. Int J Cancer 1992; 51:433-8.

30. Keller RP, Altermatt HJ, Nooter K, et al. SDZ PSC 833, a non-immunosuppressive cyclosporine: its potency in overcoming P-glycoprotein-mediated multidrug resistance of murine leukemia. Int J Cancer 1992; 50:593-7.

31. Loor F, Boesch D, Gavériaux C, Jachez B, Pourtier-Manazado A, Emmer G. SDZ 280-446, a novel semi-synthetic cyclopeptolide: in vitro and in vivo circumvention of the P-glycoprotein-mediated tumour cell multidrug resistance. Br J Cancer 1992; 65:11-8.

32. Boesch D, Muller K, Pourtier-Manzanedo A, Loor F. Restoration of daunomycin retention in multidrug-resistant P388 cells by submicromolar concentrations of SDZ PSC 833, a nonimmunosuppressive cyclosporin derivative. Exp Cell Res 1991; 196:26-32.

33. Michieli M, Damiani D, Michelutti A, et al. Restoring uptake and retention of daunorubicin and idarubicin in P170-related multidrug resistance cells by low concentration D-verapamil, cyclosporin-A and SDZ PSC 833. Haematologica 1994; 79:500-7.

34. Russo D, Marie JP, Zhou DC, et al. Evaluation of the clinical relevance of the anionic glutathione-S-transferase (GSTπ) and multidrug resistnace (mdr-1) gene coexpression in leukemias and lymphomas. Leuk Lymphoma 1994; 15:453-68.

35. Michieli M, Damiani D, Michelutti A, et al. Screening of multidrug resistance in leukemia: cell reactivity to MRK-16 correlates with anthracycline retention and sensitivity of leukemic cells. Leuk Lymphoma 1995; in press.

36. Michelutti A, Michieli M, Damiani D, et al. Overexpression of MDR-related p170 glycoprotein in chronic myeloid leukemia. Haematologica 1994; 79:200-4.

37. Pieters R, Huismans DR, Leyva A, Veerman AJP. Comparison of a rapid automated tetrazolium based (MTT)-assay with a dye exclusion assay for chemosensitivity testing in childhood leukemia. Br J Cancer 1989; 59:217-20.

38. Kuhl JS, Duran GE, Chao NJ, Sikic BI. Effects of the methoxymorpholino derivative of doxorubicin and its bioactivated form versus doxorubicin on human leukemia and lymphoma cell lines and normal bone marrow. Cancer Chemother Pharmacol 1993; 33:10-6.

39. Danesi R, Agen C, Grandi M, Nardini V, Bevilacqua G, Del Tacca M. 3'-deamino-3'-(2-methoxy-4-morpholinyl)-doxorubicin (FCE 23762): a new anthracycline derivative with enhanced cytotoxicity and reduced cardiotoxicity. Eur J Cancer 1993; 29:1560-5.

40. Barbieri B, Giuliani FC, Bordoni T, et al. Chemical and biological characterization of 4'-iodo-4'-deoxydoxorubicin. Cancer Res 1987; 47:4001-7.

41. Schott B, Vrignaud P, Ries C, Robert J, Londos-Gagliardi D. Cellular pharmacology of 4-iodo-4-deoxydoxorubicin. Br J Cancer 1990; 61:543-7.

42. Watanabe M, Komeshima N, Naito M, Isoe T, Otake N, Tsuruo T. Cellular pharmacology of MX2, a new morpholino anthracycline, in human pleiotropic drug-resistant cells. Cancer Res 1991; 51:157-61.

43. Streeter DG, Johl JS, Gordon GR, Peters JH. Uptake and retention of morpholinyl anthracyclines by adriamycin-sensitive and -resistant P388 cells. Cancer Chemother Pharmacol 1986; 16:247-52.

44. Coley HM, Twentyman PR, Workman P. 9-alkyl, morpholinyl anthracyclines in the circumvention of multidrug resistance. Eur J Cancer 1990; 26:665-7.

45. Ripamonti M, Pezzoni G, Pesenti E, et al. In vivo anti-tumor activity of FCE 23762, a methoxymorpholinyl derivative od doxorubicin active on doxorubicin-resistant tumor cells. Br J Cancer 1992; 65:703-7.

46. de Vries EGE, Zijlstra JG. Morpholinyl anthracyclines: option for reversal of anthracycline resistance. Eur J Cancer 1990; 26: 659-60.

47. Capranico G, Supino R, Binaschi M, et al. Influence of structural modifications at 3' and 4' positions of doxorubicin on the drug ability to trap topoisomrase II and to overcome multidrug resistance. Mol Pharmacol 1994; 45:908-15.

48. Mariani M, Capolongo L, Suarato A, et al. Growth-inhibitory properties of novel anthracyclines in human leukemic cell lines expressing either Pgp-MDR or at-MDR. Invest New Drugs 1994; 12:93-7.

49. Krishan A, Sridhar KS, Davila E, Vogel C, Sternheim W. Patterns of anthracycline modulation in human tumor cells. Cytometry 1987; 8:306-14.

50. Scheffer GL, Wijngaard PLJ, Flens MJ, et al. The drug resistance-related protein LRP is the human major vault protein. Nature Medicine 1995; 1:578-83.

51. Cole SPC, Bhardwaj G, Gerlach JH, et al. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 1992; 258:1650-6.

52. Ozols RF, O'Dwyer PJ, Hamilton TC, Young RC. The role of glutathione in drug resistance. Cancer Treat Rev 1990; 17:45-50.

53. Mross K, Mayer U, Langenbuch T, Hamm K, Burk K, Hossfeld D. Toxicity, pharmacokinetics and metabolism of iodoxorubicin in cancer patients. Eur J Cancer 1990; 26: 1156-62.

54. Gianni L, Viganò L, Surbone A, et al. Pharmacology and clinical toxicity of 4'-iodo-4'-deoxydoxorubicin: an example of successful application of pharmacokinetics to dose escalation in phase I trials. J Natl Cancer Inst 1990; 82:469-77.

55. Vasey PA, Bissett D, Strolin-Benedetti M, et al. Phase I clinical and pharmacokinetic study of 3'-deamino-3'-(2-methoxy-4-morpholinyl) doxorubicin (FCE 23762). Cancer Res 1995; 55:2090-6.

56. Pacciarini MA, Grandi M, Geroni C, et al. Methoxymorpholino doxorubicin (FCE 23762): preclinical characteristics and first clinical experience. In: M. Gonzales Baron, Advances in Oncology. Noesis ed. 207-213, 1995.