Overcoming multidrug resistance in Chinese hamster ovary cells in vitro by cyclosporin A (Sandimmune) and non-immunosuppressive derivatives

C. Gavériaux, D. Boesch, J.J. Boelsterli, P. Bollinger, M.K. Eberle, P. Hiestand, T. Payne, R. Traber, R. Wenger & F. Loor

Preclinical Research Department 386/125, Sandoz, CH 4002 Basel, Switzerland.

Summary
Cyclosporin A (Sandimmune) increased the in vitro susceptibility of 'parental' and 'multidrug-resistant' (MDR) Chinese hamster ovary (CHO) cell lines to three anti-tumour drugs: colchicine, daunomycin, and vincristine. Several immunosuppressive or non-immunosuppressive derivatives of cyclosporin (CsA) were compared for their ability to sensitize both parental and MDR cells to chemotherapeutic agents. Although 5-10-fold increases of sensitivity to anti-tumour drugs could be obtained for cells of the parental line with several CsA-derivatives, the largest 'gains' of sensitivity (chemosensitisation) were obtained for the cells of the MDR line and with only some of the Cs derivatives. The MDR cells employed displayed the typical MDR phenotype. However, we found no correlation between the immunosuppressive activity of Cs derivatives and their capacity to reverse MDR and all four possible combinations of these two activities could indeed be shown among the tested Cs derivatives. This study demonstrates for the first time that some immunosuppressive Cs can be devoid of chemosensitising activity.

Materials and methods

Cell lines and drugs

Chinese hamster ovary (CHO) cells were obtained from Dr V. Ling (Ontario Cancer Research Institute, Toronto, Canada) a colchicine-resistant cell line (MDR line, CHO(C5)) and the parental colchicine-sensitive cell line AUX B1 (Ling & Thompson, 1974, Bech-Hansen et al., 1975). These cell lines were grown in culture medium (αMEM medium supplemented with Asn 0.02 mg ml⁻¹, vitamins (1 x), penicillin-streptomycin 100 IU ml⁻¹, Gln 2 mM and 10% heat inactivated fetal calf serum (all from Gibco)). Colchicine (Sandoz), daunomycin (Sigma D-4885), puromycin (Sigma P-7255), vincristine (Serva 38215) and gramicidin D (Serva 24150) were prepared as stock solutions in culture medium.

Immunosuppression

The degree of immunosuppressive activity of the different Cs derivatives had been previously assessed in several in vitro and in vivo models (Hiestand & Gubler, 1988).

Proliferation assay of CHO cell lines

In preliminary experiments, we measured cell growth by methods such as 3H-thymidine uptake or a colorimetric assay (cell mass measurement by hexosaminidase content) (Koponen et al., 1982), but another colorimetric assay using MTT (Mosmann, 1983) was found to be the most convenient for screening large numbers of derivatives. This assay had also been found very 'feasible' for drug screening with panels of tumour cell lines (Alley et al., 1988).

Preliminary experiments (not shown) were performed in order to determine the culture conditions. We chose to dispense, per well, twice as many MDR cells as parental cells, so that similar cell numbers, giving optical density (OD) values in the correct range (0.8-1.4, in the colorimetric assay), were reached after a 6 day culture.

In 96-well microplates (Costar 3596), 50 µl of colchicine (or another anti-cancer drug) solution were added in culture medium in triplicate to obtain final concentrations of 0.1-30 µg ml⁻¹ for the MDR line, and 0.001-0.3 µg ml⁻¹ for the parental line. A further down-extension of the dose range was performed when necessary, i.e. when a Cs derivative was strongly decreasing the anti-cancer drug IC₅₀ (i.e. the drug dose required to reduce the final OD to 50% of control).
The Cs derivatives to be tested were dissolved at 1 mg ml\(^{-1}\) in absolute ethanol (EtOH, Merck) and were tested at 1 µg ml\(^{-1}\), with control being treated with the corresponding ethanol solvent dilutions. The cyclosporin derivatives or control solutions were added (50 µl) to each well, and mixed the 100 µl cell suspensions (4 x 10\(^{3}\) cells ml\(^{-1}\)) for the parental line and 8 x 10\(^{5}\) cells ml\(^{-1}\) for the MDR line) and colchicine solutions (50 µl) which had been added beforehand.

After a 6-day incubation at 37°C, the final cell number was measured by a colorimetric assay using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; Sigma) (Mosmann, 1983). First, 100 µl of supernatant were removed, and then to the remaining cell suspension, 10 µl of the MTT solution (5 mg ml\(^{-1}\)) were added per well and the plates incubated for 3 h at 37°C. 100 µl of solvent (butanol-2, isopropanol, HCI 1 N in volume ratio 16/8/1) were added per well and the plates shaken until complete dissolution of the formazan crystals. The OD was read at 540 nm on a plate reader (Titertek Multiskan). The extent of cell growth was represented as a function of the anti-cancer drug concentration (the growth in the absence of anti-cancer drug (Cs or solvent alone) being taken as 100%).

**Data analysis**

The anti-cancer drug IC\(_{50}\) were determined from the concentration–response curves: either in the presence of Cs (IC\(_{50}\) +), or in its absence (IC\(_{50}\) −) (but in the presence of the Cs solvent, i.e. ethanol).

The analysis of increasing slope or ‘gain of sensitivity’ brought by each Cs at 1 µg ml\(^{-1}\) were given by the ratio IC\(_{50}\) −/IC\(_{50}\) +. These measurements were performed for both cell lines (parental and MDR), for the various anti-cancer drugs assayed, and for 1 µg ml\(^{-1}\) Cs.

**Results**

In our assay system, we studied the effects of CsA and some of its derivatives at a concentration of 1 µg ml\(^{-1}\). Indeed, maximum tolerated plasma levels of CsA are in the order of 1–2 µg ml\(^{-1}\) (Kahan et al., 1983). Moreover, none of the Cs derivatives reported in this paper was toxic by itself at 1 µg ml\(^{-1}\) on parental or MDR CHO cells, no detectable effect on their growth being detected up to 3 µg ml\(^{-1}\) for all of them, and up to 10 µg ml\(^{-1}\) for most of them.

**Effect of CsA, CsH and N-phenylaminothio-carbamoyl-CsA in combination with the anti-tumour drugs on the growth of parental and MDR lines**

The parental and MDR cell lines were first compared for sensitivity to colchicine, daunomycin and vincristine, and the IC\(_{50}\) (as µg ml\(^{-1}\)) was determined for each drug. Colchicine, daunomycin and vincristine inhibited the cell growth at comparable concentrations (Figure 1, open and filled circles, for parental and MDR cells respectively). Gramicidin D and puromycin required high doses to inhibit the MDR line (IC\(_{50}\) > 50 µg ml\(^{-1}\), not shown). Figure 1 shows the effect of CsA, CsH and N-phenylaminothio-carbamoyl-CsA on the drug resistance of both CHO cell lines (the control growth in the absence of anti-tumour drug being considered as 100%), the drug–response curves being established with colchicine, vincristine and daunomycin. For the potentiation of all three anti-cancer drugs (gains of sensitivity), CsA was the most active: the vincristine IC\(_{50}\) and daunomycin IC\(_{50}\) of MDR cells in the presence of CsA even fell below the vincristine IC\(_{50}\) and daunomycin IC\(_{50}\) of parental line in the absence of CsA. CsA was also effective on the parental line itself, the parental gains and MDR 50% gains being respectively 10 and 14 for colchicine, 11 and 47 for daunomycin, and 22 and 77 for vincristine. The N-phenylaminothio-carbamoyl-CsA was essentially inactive, the gains of sensitivity being below 2. CsH, an immunologically inactive derivative of CsA, was weakly active in our assay, giving parental gains and MDR gains of, respectively, 4 and 2.5 for daunomycin, 4.5 and 2 for vincristine, and 6 and 1.2 for colchicine.

**Effect of some Cs derivatives on resistance to colchicine, vincristine and daunomycin**

The IC\(_{50}\) ranges for colchicine, vincristine and daunomycin in our assay were the following, respectively (in ng ml\(^{-1}\)):

- 18.5–53, 50–175 and 14–23.5 for parental line, and
- 980–3,100, 660–5,900 and 500–2,100 for the MDR line.

The ability of some Cs derivatives with different immunosuppressive powers to sensitize parental and MDR CHO cells to the three anti-tumour drugs is shown in Table I for colchicine, in Table II for vincristine and in Table III for daunomycin. There is a good agreement between the effects of these 15 Cs derivatives on colchicine, vincristine and daunomycin resistance. Some Cs derivatives could overcome colchicine, vincristine and daunomycin resistance, thus giving an IC\(_{50}\) + for MDR cells similar to the IC\(_{50}\) of parental cells: CsA, CsG, (Me-Ala\(^{-}\))CsA, O-Acetyl-CsA, (O-Bu-Ser\(^{-}\))Cs, (Me-Ile\(^{-}\))Cs and (3′-deoxy-3′-oxo-MeBm\(^{-}\))Cs.

No correlation was found between the immunosuppressive and MDR sensitising properties of these 15 derivatives. Some Cs were both immunosuppressive and active in MDR (CsA, CsG, (Me-Ala\(^{-}\))Cs), some were immunosuppressive but inactive in MDR ((D-Ser\(^{8}\))Cs, (dhBmt-1,α-S-Me-Sar\(^{3}\),Val\(^{7}\))Cs, (8′-Methoxy-dh-MeBm\(^{7}\))Cs, dihydroCS), some were non-immunosuppressive but active in MDR (O-acetyl-CsA,
| Cs derivative | Parental No. | IC₅₀ parental cells (ng ml⁻¹) | IC₅₀ MDR cells (ng ml⁻¹) | Gain | IC₅₀ - | Gain | P | Gain IMMUNOSUPPRESSION |
|---------------|--------------|-------------------------------|--------------------------|------|--------|------|---|------------------------|
| CsA           | 9            | 3.0(0.9)                     | 23.0(170)                | 7.7  | 0.001  | 8.6  | ++ + + + + + + +       |
| CsG           | 3            | 3.7(0.3)                     | 27.5(7)                  | < 0.001 | 72.7  | + + + + + +            |
| (Me-Ala₃)-Cs  | 2            | 4.1(0.1)                     | 46(5.6)                  | < 0.001 | 41.3  | + + + + + +            |
| (D-Ser²)-Cs   | 2            | 4.5(1.3)                     | 1800(280)                | > 0.1   | 1.1    | + + + + + + +           |
| (dhBmt-Ix-S-Me-Sar²,Val²)-Cs | 2 | 3.5(1.7) | 7400(80) | > 0.001 | 2.4 | + + + + + + + + +  |
| (8'Methoxy-dh-Mebmt²)-Cs | 2 | 5.9(0.1) | 1620(110) | < 0.05  | 1.1   | + + + + + + + + +  |
| dihydroCs    | 3            | 3.1(0.8)                     | 990(300)                 | > 0.1   | 2.0    | + + + + + + + + + + + |
| O-Acetyl-CsA | 3            | 3.8(0.9)                     | 71(11)                   | < 0.001 | 31.7  | + + + + + + + + + + + |
| (O-Bu-Ser²)-Cs | 5   | 2.6(0.6) | 2250(460) | < 0.001 | 91.9  | + + + + + + + + + + + |
| (Me-Ble³)-Cs  | 4            | 2.8(0.9)                     | 2050(100)                | < 0.001 | 35.2  | + + + + + + + + + + + |
| (3'-deoxy-3'-oxo-Mebmt²)-Cs | 3 | 3.3(0.7) | 1733(104) | < 0.001 | 86.0  | + + + + + + + + + + + |
| (Pro³)-Cs    | 2            | 3.3(0.6)                     | 257(60)                  | < 0.001 | 7.1    | + + + + + + + + + + + |
| (O-Acetyl-Thr²)-Cs | 6 | 3.6(0.8) | 550(340) | < 0.001 | 3.9    | + + + + + + + + + + + |
| CsH           | 4            | 5.3(0.9)                     | 1630(100)                | > 0.1   | 1.3    | + + + + + + + + + + + |
| N-phenyl-aminothio-carbamoyl-CsA | 3 | 20.7(2.9) | 2420(600) | > 0.1   | 1.2    | + + + + + + + + + + + |

*Parental and MDR line cells (400 cells per well and 800 cells per well, respectively) were incubated with colchicine and the Cs derivatives for 6 days. Cell proliferation was measured by the MTT assay. The IC₅₀Cs and IC₅₀MDR were the colchicine IC₅₀ in the presence and absence of Cs at 1 μg ml⁻¹ (mean ± s.d. of indicated independent experiments). IC₅₀ differences were calculated by Student’s t test versus the means of all EtOH solvent controls: for parental cells, with all Cs derivatives P < 0.001. *Number of independent experiments (each in triplicate). +Gain of sensitivity was defined by the ratio IC₅₀MDR/IC₅₀Cs. #N-phenyl-aminothio-carbamoyl-CsA (P < 0.05) versus the EtOH solvent control.

| Cs derivative | Parental No. | IC₅₀ parental cells (ng ml⁻¹) | IC₅₀ MDR cells (ng ml⁻¹) | Gain | IC₅₀ - | Gain | P | Gain IMMUNOSUPPRESSION |
|---------------|--------------|-------------------------------|--------------------------|------|--------|------|---|------------------------|
| CsA           | 5            | 4.6(1.5)                     | 65(45)                   | 16.1 | 0.001  | 37.5 | + + + + + + + + + + + |
| CsG           | 6            | 4.6(1.6)                     | 38.3(21)                 | 16.2 | 0.001  | 59.0 | + + + + + + + + + + + |
| (Me-Ala₃)-Cs  | 2            | 5.7(0)                       | 57.5(5)                  | 13.2 | 0.001  | 31.7 | + + + + + + + + + + + |
| (D-Ser²)-Cs   | 2            | 5.4(1.9)                     | 1800(90)                 | 15.7 | 0.001  | 1.1  | + + + + + + + + + + + |
| (dhBmt-Ix-S-Me-Sar²,Val²)-Cs | 2 | 6.7(0.4) | 1315(21) | < 0.01  | 1.4    | + + + + + + + + + + + |
| (8'Methoxy-dh-Mebmt²)-Cs | 2 | 16.0(7) | 1725(35) | < 0.01  | 1.1    | + + + + + + + + + + + |
| dihydroCsC    | 2            | 4.9(0.4)                     | 840(295)                 | 17.3 | 0.001  | 2.3  | + + + + + + + + + + + |
| O-Acetyl-CsA  | 2            | 7.4(0.9)                     | 58(3)                    | 10.7 | 0.001  | 3.9  | + + + + + + + + + + + |
| (O-Bu-Ser³)-Cs | 5   | 7.5(0.3) | 50(11) | < 0.001  | 36.9 | + + + + + + + + + + + |
| (Me-Ble³)-Cs  | 2            | 5.6(0.1)                     | 51(5.2)                  | 13.4 | 0.001  | 35.4 | + + + + + + + + + + + |
| (3'-deoxy-3'-oxo-Mebmt²)-Cs | 2 | 6.1(0.4) | 92.5(56) | < 0.001  | 19.9 | + + + + + + + + + + + |
| (Pro³)-Cs    | 2            | 5.7(0.1)                     | 270(28)                  | 12.3 | 0.001  | 6.8  | + + + + + + + + + + + |
| (O-Acetyl-Thr²)-Cs | 2 | 6.1(0.3) | 360(57) | < 0.001  | 5.3  | + + + + + + + + + + + |
| CsH           | 2            | 23.8(4.6)                    | 350(355)                 | 7.1  | 0.001  | 1.1  | + + + + + + + + + + + |
| N-phenyl-aminothio-carbamoyl-CsA | 3 | 54(5.1) | 1050(377) | < 0.01  | 1.1    | + + + + + + + + + + + |

Same legend as for Table I. IC₅₀ differences were calculated by Student’s t test. For parental cells, with all Cs derivatives P < 0.001 versus the EtOH solvent control.
The use of cyclosporin A to overcome multidrug resistance of tumour cells has been reported by several investigators. Slater et al. (1986b) described some effect of CsA at 3.3 μg ml⁻¹ on vincristine and daunorubicin susceptibility of MDR acute lymphatic leukaemia cells but not of parental cells, whereas little effect was found for daunorubicin suscepti-

bility of parental and MDR Ehrlich ascites carcinoma cells (about a 2-fold increase in sensitivity) (Slater et al., 1986a). Meinerz et al. (1987) found some effect of CsA at 1 μg ml⁻¹ in drug sensitive Ehrlich ascites carcinoma and murine hepatoma 129 cell lines, although this effect was small com-

pared to our experiments. Twentyman et al. (1987) first indicated some agreement between the immunosuppressive and the MDR sensitising properties of four Cs derivatives, but they later demonstrated (1988), using further non-immunosuppressive derivatives, that these two properties could be dissociated.

Using similar assay systems and cell lines whose MDR phenotype dependence on Pgp-mediated efflux is well established (Kartner et al., 1983), we found that CsA decreased resistance to all three drugs colchicine, vincristine and daunorubicin (Figure 1): it not only decreased the IC₅₀ of the drugs in MDR cells, but also in parental cells. Experiments in progress, using several other cell lines from both parental and MDR lines are available, show that chemosensitisation of 'parental' cells by CsA (or Cs-derivatives) is not a common property (results not shown). The parental CHO cell line has IC₅₀ in the orders of 30 ng ml⁻¹ for colchicine, 80 ng ml⁻¹ for vincristine and 20 ng ml⁻¹ for daunorubicin, which is about 10-fold higher than IC₅₀ measured for other parental cells as well as for normal cells. Perhaps the cells of the parental CHO line express small amounts of Pgp, confer-

ring upon them a weak multidrug resistance, thus making them somewhat susceptible to chemosensitisation down to the normal IC₅₀ limit observed with these drugs in other cells which do not contain Pgp.

Interestingly, our experiments showed differential effects of CsA on MDR attenuation depending on the anti-tumour drug tested. Indeed, the MDR gains for vincristine, daunorubicin and colchicine were 77, 47 and 14 respectively in experiments run in parallel (Figure 1). Thus, CsA-mediated chemosensitisation was not as strong for colchicine as for daunorubicin and vincristine. CsA reversed vincristine and daunorubicin resistance of MDR cells completely, but only part of their colchicine resistance. Such differential chemosen-

sitisation on various anti-cancer drugs had already been observed with quinacrine (Inaba & Maruyama, 1988) and with the calcium channel blocker Verapamil (Beck et al., 1986).

The non-immunosuppressive cyclosporin, CsH₄, was inac-

tive at 1 μg ml⁻¹ in reversing colchicine and vincristine resistance but slightly active for daunorubicin resistance of MDR cells, extending the data of Twentyman et al. (1987) on the decrease of adriamycin resistance with 5 μg ml⁻¹ of this compound. However, it slightly potentiated the inhibitory effects of all three drugs on parental cells. The latter characteristic may be due to the easier neutralisation by CsH₄ of the presumed lower levels of Pgp present in parental cells.

Since the MDR cells contain much more Pgp than the parental cells (Van der Bliek et al., 1986; Scheper et al., 1988), it can indeed be expected that hitherto inactive Cs derivatives, endowed with low Pgp-neutralising capacity, will show stronger effects on the parental cells than on the MDR cells. Some other Cs derivatives ((D-Ser⁸)-Cs and (8' Methoxy-dh-Mebmt)-Cs) even gave detectable gains of sen-

sitivity with the low-Pgp parental cells whereas no effect was found with high-Pgp MDR cells.

We found no correlation between the immunosuppressive activity and the MDR-neutralising activity of more than 120 Cs derivatives (and of about 100 structurally related molecules) tested so far. As shown here for 15 derivatives tested on CHO cells in which the MDR property is definitely caused by Pgp-mediated drug efflux, four Cs derivative categories can be defined, some sharing both immunosup-

pressive and MDR-neutralising activities, some showing only one and some others being devoid of both activities. We thus confirm and extend the study of Twenty-

man (1988), who showed the chemosensitising properties of poorly or non-immunosuppressive Cs derivatives on drug resistant H69 cells, as well as those of Hait et al. (1987) and Chambers et al. (1988), who mentioned some chemosensitis-

ing activity of one non-immunosuppressive Cs.

For the four non- or weakly immunosuppressive Cs derivatives used by Twentyman (1988) on parental and MDR H69 cells, the decreasing order of efficacy for chemosensitisation towards adriamycin and vincristine was the following: O-Acetyl-CsA>(Me-Ile⁷)-Cs>CsA>(Me-Ala⁶)-Cs. In their hands, and considering only non-immunosuppressive Cs derivatives, (Me-Ile⁷)-Cs and O-acetyl-CsA gave the highest chemosensitisation to daunorubicin whereas (O-tBu-Ser²)-Cs and (Me-Ile⁷)-Cs gave the highest chemosensitisation to vincristine, and finally (O-tBu-Ser²)-Cs and (3'-deoxy-3'-oxo-

MeBmt¹)-Cs gave the highest chemosensitisation to col-

chicine. From our results and those of Twentyman (1988), it thus appears that a given Cs derivative which may be the best to overcome resistance to one anti-tumour drug is not necessarily as effective in overcoming the resistance to several anti-tumour agents.

Since immunosuppressive Cs derivatives bind to the 'intracellular receptor' cyclophilin, whereas non-immuno-

suppressive Cs do not (Handschumacher et al., 1984; Quesniaux et al., 1987), cyclophilin appears not to be involved in MDR. Naito and Tsuruo (1989) have demonstrated that CsA was as effective as vinblastine for the inhibition of the high affinity binding of vincristine to plasma membranes of MDR K562 cells. Whether the MDR active and inactive Cs actually decrease the anti-tumour drug efflux out of the cell and bind to the Pgp might help to elucidate the mechanism of action of Cs in MDR. Patients who require immunosuppression for organ transplantation or autoimmunity treatment might benefit from being treated with Cs derivatives which do not affect the function of their normal Pgp. In this regard, it is important that some immunosuppressive Cs derivatives (O-tBu-Ser²S, Me-Ser³, Val²³-Cs and (8'Methoxy-dh-Mebmt)-Cs) are completely devoid of MDR sensitising properties.

The prime interest for clinical cancer therapy will be the identification of non-immunosuppressive Cs derivatives with very potant MDR neutralising activity, good pharmacokinetic properties in vivo, but that are devoid of toxic effect on normal cells.

We are very grateful to Dr Jean Borel who drew our attention to this new property of cyclosporin.
References

ALLEY, M.C., SCUDIERO, D.A., MONKS, A. & 7 others (1988). Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res., 48, 589.

BECH-HANSEN, N.T., TILL, J.E. & LING, V. (1975). Pleiotropic properties of colchicine-resistant CHO cells: cross-resistance and collateral sensitivity. J. Cell. Physiol., 88, 23.

BECK, W.T., CURTAIN, M.C., LOOK, T. & ASHUMUM, R.A. (1986). Reversal of vinca alkaloid resistance but not multiple drug resistance in human leukemic cells by verapamil. Cancer Res., 46, 778.

CHAMBERS, S.K., HAIT, W.N., HARDING, M.W. & HANDSCHUMACHER, R.E. (1988). Cyclosporin A and non-immunosuppressive homolog can sensitize parent and multidrug resistant ovarian cell lines to doxorubicin. Proc. AACR, 29, 313 (abstract).

GERLACH, J.H., KARTNER, N., BELL, D.R. & LING, V. (1986). Multidrug resistance. Cancer Surv., 5, 25.

GOTTMESMAN, M.M. & PASTAN, I. (1988). Resistance to multiple chemotherapeutic agents in human cancer cells. TIPS, 9, 54.

HAIT, W.N., STEIN, J.M., KOLETSKY, A.J., SLATER, I.M., HARDING, M.W. & HANDSCHUMACHER, R.E. (1987). Modulation of doxorubicin (DOX) resistance by cyclosporin A (CsA) and a non-immunosuppressive homolog. Proc. AACR, 28, 298, (abstract).

HANDSCHUMACHER, R.E., HARDING, M.W., RICE, J., DRUGGE, R.J. & SPEICHER, D.W. (1984). Cyclophilin: a specific cytosolic binding protein for cyclosporin A. Science, 226, 544.

HIESTAND, P.C. & GUBLER, H.U. (1988). Cyclosporins: immunopharmacologic properties of natural cyclosporin. In Handbook of Experimental Pharmacology, Bray, M.A. & Morley, J. (eds) Springer-Verlag, Berlin p. 487.

INABA, M. & MARUYAMA, E. (1988). Reversal of resistance to vincristine in P388 leukemia by various polycyclic clinical drugs, with a special emphasis in quinacrine. Cancer Res., 48, 2064.

KAHAN, B.D., RIED, M. & NEWBURGER, J. (1983). Pharmacokinetics of cyclosporine in human renal transplantation. Transpl. Proc., 15, 446.

KARTNER, N., RIORDAN, J.R. & LING, V. (1983). Cell surface P-Glycoprotein associated with multidrug resistance in mammalian cell lines. Science, 221, 1285.

KOPONEN, M., GRIEDER, A. & LOOR, F. (1982). The effects of cyclosporins on the cell cycle of T-lymphoid cell lines. Exp. Cell Res., 140, 237.

LING, V. & THOMPSON, L.H. (1974). Reduced permeability in CHO cells as a mechanism of resistance to colchicine. J. Cell. Physiol., 83, 103.

MEADOR, J., SWEET, P., STUPECKY, M. & 4 others (1987). Enhancement by cyclosporin A of daunorubicin efficacy in Ehrlich ascites carcinoma and murine hepatoma 129. Cancer Res., 47, 6216.

MOSMANN, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assays. J. Immunol. Methods, 65, 55.

NAITO, M. & TSURUO, T. (1989). Competitive inhibition by verapamil of ATP-dependent high affinity vincristine binding to the plasma membrane of multidrug-resistant K562 cells without calcium involvement. Cancer Res., 49, 1452.

OSIEKA, R., SEEBER, S., PANNENBCKER, R., SOLL, D., GLATTE, P. & SCHMIDT, C.G. (1986). Enhancement of etoposide-induced cytotoxicity by cyclosporin A. Cancer Chemother. Pharmacol., 18, 198.

QUESNIAUX, V.F.J., SCHREIER, M.H., WENGER, R.M., HIRST, P.C., HARDING, M.W. & VAN REGENMORTEL, M.H.V. (1987). Cyclophilin binds to the regions of cyclosporin involved in its immunosuppressive activity. Eur. J. Immunol., 17, 1359.

SCHEPER, R.J., BULTE, J.W.M., BRAKKE, J.G.P. & 6 others (1988). Monoclonal antibody JSB-1 detects a highly conserved epitope on the P-glycoprotein associated with multi-drug-resistance. Int. J. Cancer, 42, 389.

SLATER, L.M., SWEET, P., STUPECKY, M., WETZEL, M.W. & GUPTA, S. (1986a). Cyclosporin A corrects daunorubicin resistance in Ehrlich ascites carcinoma. Br. J. Cancer, 54, 235.

SLATER, L.M., SWEET, P., STUPECKY, M. & GUPTA, S. (1986b). Cyclosporin A reverses vincristine and daunorubicin resistance in acute lymphatic leukemia in vitro. J. Clin. Invest., 77, 1405.

STARK, G.R. (1986). Progress in understanding multidrug resistance. Nature, 324, 407.

TWENTYMAN, P.R., FOX, N.E. & WHITE, D.J.G. (1987). Cyclosporin A and its analogues as modifiers of adriamycin and vincristine resistance in a multi-drug-resistant human lung cancer cell line. Br. J. Cancer, 56, 55.

TWENTYMAN, P.R. (1988). Modification of cytotoxic drug resistance by non-immuno-suppressive cyclosporins. Br. J. Cancer, 57, 25.

UEDA, K., CARDARELLI, C., GOTTMESMAN, M.M. & PASTAN, I. (1987) Expression of a full-length cDNA for the human 'MDR' gene confers resistance to colchicine, doxorubicin, and vinblastine. Proc. Natl Acad. Sci. USA, 84, 3004.

VAN DER BLIEK, A.M., VAN DER VELDE-KOERTS, T., LING, V. & BORST, P. (1986). Overexpression and amplification of five genes in a multidrug-resistant Chinese hamster ovary cell line. Mol. Cell. Biol., 6, 1671.