Pergamon

European Journal of Cancer Vol. 32A, No. 5, pp. 857–861, 1996

Printed in Great Britain
0959-8049/96 $15.00+0.00

0959-8049(95)00661-3

Original Paper

Modulation of P-glycoprotein Mediated Drug Accumulation in Multidrug Resistant CCRF VCR-1000 Cells by Chemosensitisers

R. Boer, V. Gekeler, W.-R. Ulrich, P. Zimmermann, W. Ise, A. Schödl and S. Haas

Byk Gulden, Byk Gulden-Strasse 2, D-78467 Konstanz, Germany

P-glycoprotein (PGP) mediated transport of cytostatic drugs out of resistant cancer cells is a major cause of experimental and probably also of clinical multidrug resistance, which often leads to treatment failure during chemotherapy. The broad substrate specificity of PGP strongly restricts effective chemotherapy and diminishes the patients' prognosis. Inhibition of PGP's pumping function by chemosensitisers is one way to restore cellular responsiveness to otherwise ineffective cytostatics. Clinical trials with several chemosensitisers are under way. To date, it is not clear whether a certain chemosensitiser potentiates the action of different cytostatic drugs, transported by PGP equally well, or whether the chemosensitising potency is dependent on the cytostatic drugs used. Therefore, we compared the effects of five potent chemosensitisers on cellular accumulation using [^{3}H]daunomycin, [^{3}H]vincristine and rhodamine-123 as substrates for PGP. The acridonecarboxamide derivative GF 120918 was the most potent compound and a half-maximal effect was seen at concentrations ranging from 5 nM for rhodamine-123 accumulation to 14 and 19 nM for [^{3}H]vincristine or [^{3}H]daunomycin accumulation, respectively. The new chemosensitiser B9203-016 was slightly less effective than GF 120918 in all three test systems. Dexniguldipine was of intermediate potency with half-maximal effects at concentrations between 62 and 194 nM. The cyclic undecapeptide SDZ PSC 833 showed somewhat lower potency ranging from 151 to 331 nM. Cyclosporin A was less potent than SDZ PSC 833. Furthermore, enhancement of drug accumulation produced by each chemosensitiser was similar, regardless of which PGP substrate was measured, that is, the rank order of potency to increase accumulation was the same in each of the assays used. Our data point to similar, if not identical, mechanisms of drug transport by PGP and inhibition of drug transport by chemosensitisers at least for the substrates rhodamine-123, vincristine and daunomycin.

Key words: multidrug resistance, P-glycoprotein, chemosensitisers, drug transport
Eur J Cancer, Vol. 32A, No. 5, pp. 857–861, 1996

INTRODUCTION

P-GLYCOPROTEIN (PGP) is an ATP-driven efflux pump for a variety of cytostatics and other drugs, and causes the development and manifestation of multidrug resistance in animal and human cancer cells [1, 2]. The protein consists of 1280 amino acids and has a molecular weight of approximately 170 kDa. Expression of the protein in plasma membranes of cancer cells leads to increased drug efflux with a concomitant decrease of intracellular drug content. PGP shows broad substrate specificity, resulting in transport of structurally different substrates, such as vinca alkaloids, anthracyclines, taxanes, epipodophyllotoxins, rhodamine-123 and other compounds [3]. PGP mediated drug transport has been shown with radiolabelled cytostatics [4], or by using the fluorescence of anthracyclins [5] as marker signals. Additionally, owing to the strong fluorescence of the cationic dye rhodamine-123, this compound is a convenient and often used substrate for monitoring PGP mediated drug transport [6].

Inhibition of PGP by chemosensitisers is one approach for overcoming multidrug resistance and in restoring sensitivity of otherwise unresponsive tumour cells or tumours. Chemosensitising compounds are of a similar structural diversity as the transported substrates and include older compounds such

Correspondence to R. Boer.
Revised 13 Sep. 1995; accepted 23 Nov. 1995.

as verapamil, quinidine, tamoxifen, nifedipine and others [7, 8]. Chemosensitisers of the second and third generation were designed for higher potency and reduced side-effects, and are represented by compounds such as SDZ PSC-833 [9], dexniguldipine [10, 11], S9788 [12], GF 120918 [13] and others. There exists a huge amount of *in vitro* data in various test systems and cell lines, showing chemosensitisation towards different cytostatics. Nevertheless, it is unclear, so far, whether the chemosensitising potency of a particular chemosensitiser is the same for different cytostatics or compounds, transported by PGP. This is not only important for the understanding of the biochemical mechanism of drug transport by PGP, but is also relevant for the clinical development of chemosensitisers. Therefore, we compared the effects of the chemosensitisers dexniguldipine, SDZ PSC-833, cyclosporin A, GF 120918 and a new pyridine derivative, B9203-016, on the accumulation of different substrates for PGP. We have chosen SDZ PSC-833, cyclosporin A and dexniguldipine as these compounds are in clinical trials. B9203-016 and GF 120918 were included because of their high potency [13]. Figure 1 shows the structure of the chemosensitiser B9203-016. Vincristine and daunomycin were chosen for their clinical relevance as cytostatics, and rhodamine-123 was included because this compound is often used as a model substrate for the investigation of PGP mediated drug transport and for the fast screening of new chemosensitisers.

MATERIALS AND METHODS

Materials

Rhodamine-123 was obtained from Sigma, Deisenhofen, Germany. [^{3}H]Vincristine (6–9 Ci/mmol) and [^{3}H]daunomycin (1.26 Ci/mmol) were obtained from Amersham, Braunschweig, Germany.

3-[8-(4,4-diphenyl-piperidin-1-yl)-octanoyl]-2-methyl-4-(3-nitro-phenyl)-7,8-dihydro-6H-quinolin-5-one-hydrochloride (B9203-016), dexniguldipine–HCl and N-{4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl}-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF 120918) were synthesised at Byk Gulden. SDZ PSC-833 was a kind gift from Sandoz/Basel (Switzerland). All chemosensitisers were dissolved 200-fold the final concentration in DMSO and diluted 200-fold by addition of 2.5 or 5 μl to the test mixture.

Cell lines and cell culture

The human T-lymphoblastoid cell line CCRF-CEM was obtained from the American Type Culture Collection (Rockville, Maryland, U.S.A.). The selection of the multidrug resistant CCRF-CEM subline CCRF VCR-1000 has been reported elsewhere [14]. Cells were propagated at 37°C in a 5% CO_2 atmosphere in RPMI 1640 medium containing 10% FCS, 2 mM glutamine and 50 μg/ml gentamicin. Stock cultures of CCRF VCR-1000 cells were grown in the presence of 1000 ng/ml vincristine.

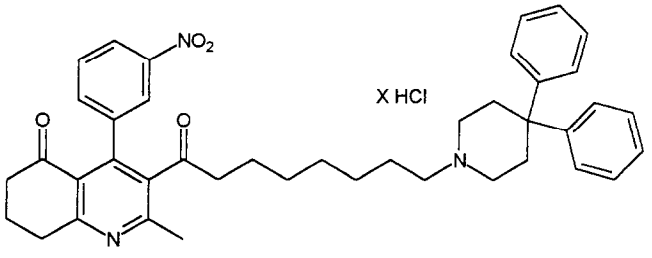

Figure 1. Structure of B9203-016.

Cellular drug sensitivity assays

Cellular drug sensitivity was tested using a modification of the original tetrazolium based colorimetric MTT-assay [15] as described earlier [16].

Rhodamine-123 accumulation

Rhodamine-123 accumulation was performed essentially as described earlier [11].

[^{3}H] *Vincristine and* [^{3}H] *daunomycin accumulation*

Cells were harvested by centrifugation at 100*g* for 10 min. Cells (1×10^6) were incubated with 10 μM daunomycin or vincristine in the absence and presence of varying concentrations of chemosensitisers. To monitor drug accumulation, [^{3}H]vincristine and [^{3}H]daunomycin was included (5×10^5–5×10^6 dpm). Cells were incubated in a total volume of 0.5 ml for 90 min at 37°C, and then rapidly cooled to 0°C in an ice water bath. Cells were centrifuged at 1000*g* for 5 min and were washed once with 1 ml of ice-cold phosphate buffered saline, pH 7.4. Cells were again centrifuged and the supernatant was carefully decanted. Cells were resuspended in 100 μl of water and cellular radioactivity was determined by liquid scintillation counting. All points were measured in duplicate.

RESULTS

The cellular vincristine, daunomycin and rhodamine-123 sensitivity of the human PGP expressing T-lymphoblastoid cell line CCRF VCR-1000 and the parental, drug sensitive cell line CCRF-CEM was determined by measuring cellular proliferation in a 3-day colorimetric tetrazolium based MTT-assay. The multidrug resistant human T-lymphoblastoid cell line CCRF VCR-1000 used in this study was selected from the parental CCRF-CEM cell line. It shows an approximately 20-fold higher expression of the *MDR1* gene at the mRNA level compared to the CCRF-CEM cell line which was detected by cDNA polymerase chain reaction (data not shown). The cell line CCRF VCR-1000 showed a 10220-fold resistance to vincristine, a 70-fold resistance to daunomycin and a 270-fold resistance to rhodamine-123 in comparison to the sensitive CCRF-CEM cell line.

All chemosensitisers increased the accumulation of the three substrates rhodamine-123, [^{3}H]vincristine and [^{3}H]daunomycin 3–5-fold in multidrug resistant CCRF VCR-1000 cells. This increase shows a clear dose dependency, and, at high compound concentrations, a saturation of the chemosensitising effect was seen. The only exception was the modulation of rhodamine-123 accumulation by cyclosporin A, which did not saturate near the level of the sensitive cells, but led to a further 2–3-fold increase of cellular rhodamine-123 content at concentrations <5 μM. In Figure 2, the increase in accumulation of rhodamine-123, [^{3}H]vincristine and [^{3}H]daunomycin in the presence of increasing concentrations of dexniguldipine is shown. Sigmoid dose–response curves were fitted to the data, and the concentration leading to half-maximal chemosensitisation (EC_{50}-value) is used to describe the potency of the compounds. Similar log EC_{50}-values (pEC_{50}-values) of 6.71, 6.91 and 7.21 were found for dexniguldipine with regard to rhodamine-123, [^{3}H]daunomycin and

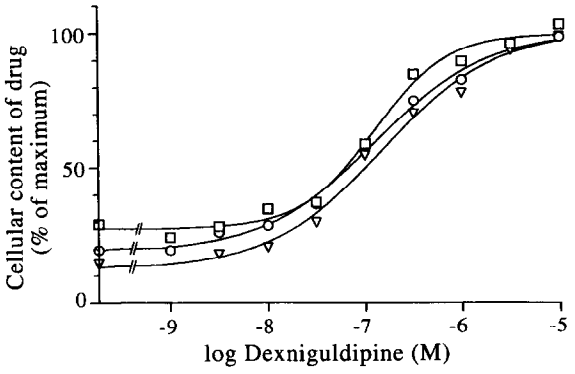

Figure 2. Dose-dependent increase in cellular content of (▽) rhodamine-123, (○) [^{3}H]vincristine and (□) [^{3}H]daunomycin in the multidrug resistant human T-lymphoblastoid cell line CCRF VCR-1000 by dexniguldipine. pEC_{50}-values for the experiments shown were 6.82, 6.89 and 6.91 for rhodamine-123, [^{3}H]vincristine or [^{3}H]daunomycin accumulation, respectively.

[^{3}H]vincristine accumulation. The new chemosensitiser B9203-016 modulated the accumulation of the three model substrates with almost identical but higher potency showing pEC_{50}-values around 7.5. The cyclic undecapeptide SDZ PSC-833 was of similar potency as dexniguldipine. Cyclosporin A was approximately 3-fold less effective than SDZ PSC-833. The acridone carboxamide derivative GF 120918 was the most potent compound in all three test systems and pEC_{50}-values varied between 7.7 for [^{3}H]daunomycin and 8.3 for rhodamine-123 accumulation. In Table 1, the pEC_{50}-values for all chemosensitisers are given for the three substrates of P-glycoprotein. The influence of these chemosensitisers on the accumulation of [^{3}H]vincristine, [^{3}H]daunomycin and rhodamine-123 in the sensitive cell line CCRF-CEM without significant P-glycoprotein expression was also investigated. All chemosensitisers were tested up to concentrations of 1 μM, except for cyclosporin A, which was also tested at 10 μM. No significant change in intracellular [^{3}H]vincristine, [^{3}H]daunomycin and rhodamine-123 concentrations were found in the presence of the different modulators. However, at cyclosporin A concentrations >5 μM, a similar increase in rhodamine-123 accumulation as found for the resistant cells was also seen in the parental cell line. This increase is therefore not due to the presence of P-glycoprotein.

To assess the question of whether the elevated accumulation of P-glycoprotein substrates by chemosensitisers translates into an increased cytotoxicity on the cellular level, sensitivity of CCRF VCR-1000 cells towards vincristine was determined in the absence or presence of various concentrations of chemosensitisers, respectively. Cellular proliferation was measured by the use of the colorimetric MTT-test. In the presence of chemosensitisers, there was a dose-dependent increase in sensitivity towards vincristine and at high concentrations the sensitivity of the parental CCRF-CEM cell line was re-established. Figure 3 shows the reversal of vincristine resistance in CCRF VCR-1000 cells at a concentration of 0.3 μM of chemosensitiser, a concentration which should be achievable clinically for all compounds. The most potent compound was GF-120918, resulting in a 67% reversal of resistance at this concentration. High potency was also seen with SDZ PSC-833, while cyclosporin was a weak chemosensitiser at 0.3 μM. Dexniguldipine and B9203-012 were of intermediate potency. The concentration of chemosensitiser resulting in half-maximal reversal of vincristine resistance are given in Table 1.

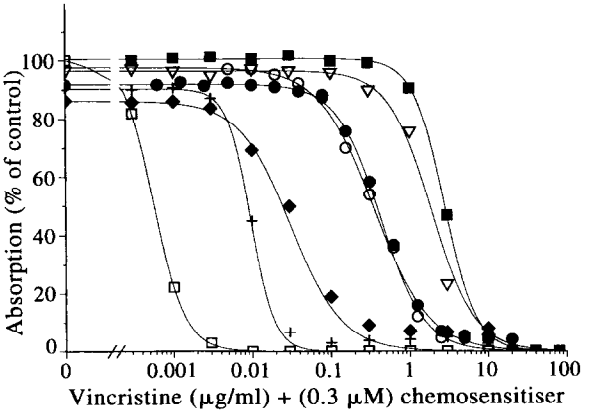

Figure 3. Cytotoxicity of vincristine in multidrug resistant CCRF VCR-1000 cells (■) and drug sensitive parental CCRF-CEM cells (□) in the absence (■, □) and presence of 0.3 μM of (+) GF 120918, (◆) SDZ PSC-833), (○) dexniguldipine, (●) B9203-016 and (▽) cyclosporin A.

Table 1. Potency of chemosensitisers to restore cellular accumulation of P-glycoprotein substrates and chemosensitising potency towards vincristine in multidrug resistant CCRF VCR-1000 cells. Log concentrations (in mol/l) with standard deviations leading to a half-maximal increase in cellular content (pEC_{50}) of rhodamine-123, [^{3}H]vincristine and [^{3}H]daunomycin, and log concentrations in mol/l leading to half-maximal reversal of vincristine resistance are given

Compound	Rhodamine-123 accumulation	[^{3}H]Daunomycin accumulation	[^{3}H]Vincristine accumulation	Chemosensitising potency
Dexniguldipine	6.71 ± 0.26	6.91 ± 0.21	7.21 ± 0.13	6.0
B9203-016	7.57 ± 0.20	7.51 ± 0.10	7.48*	6.2
SDZ PSC-833	6.67 ± 0.12	6.82 ± 0.10	6.48*	6.3
Cyclosporin A	5.93 ± 0.07†	6.36 ± 0.21	n.d.	5.6
GF 120918	8.30 ± 0.19	7.72 ± 0.20	7.84 ± 0.27	8.1

*Mean of two experiments which differed by not more than 0.3 from each other. †Owing to a large increase in rhodamine-123 content at concentrations above 5 μmol/l, which is also seen in the sensitive cell line CCRF-CEM without significant P-glycoprotein expression, dose–response curves could not be constructed. The value was therefore estimated from the rightward shift of the dose–response curves compared to GF 120918 and B9203-016.

n.d., not determined.

DISCUSSION

This study was performed in order to determine whether drug transport of different drugs by PGP is inhibited in a similar manner by a particular chemosensitiser or whether inhibition of drug transport by chemosensitisers is dependent on the transported drug. The cell line CCRF VCR-1000 shows a 20-fold higher PGP expression compared to the drug-sensitive CCRF-CEM cell line. A weak PGP positive signal at the detection limit has been seen in Western blots with CCRF-CEM cells using the PGP-specific antibody C219, but this low expression seems to be of minor relevance for our drug accumulation studies. This assumption is supported by the fact that we did not find any modulation of drug accumulation in CCRF-CEM by all chemosensitisers tested, except using cyclosporin A together with rhodamine-123 as a substrate. The effects of cyclosporin A at high concentrations (>5 μM) in parental CCRF-CEM cells and the resistant CCRF VCR-1000 cells could be due to a non-specific membrane perturbing activity of the drug. However, we cannot totally exclude the possibility that other drug transporters, which are inhibited by cyclosporin A, are also present in these cell lines. It has been shown by others that cyclosporin A acts as a non-specific drug transport inhibitor, also blocking other putative drug transporting proteins [17, 18] which may be expressed in sensitive and resistant cell lines beside P-glycoprotein. For instance, expression of the MRP-gene (multidrug resistance associated protein) was recently demonstrated by cDNA-PCR in CCRF-CEM cells [19]. However, no expression of MRP was found in either CCRF-CEM cells or in CCRF VCR-1000 cells by Western blotting, at the protein level, by using MRP-specific antibodies (Gekeler *et al.*, unpublished results). Therefore, data obtained with the cell line CCRF VCR-1000 are likely to be representative for PGP-mediated multidrug resistance. The PGP expressed in CCRF VCR-1000 cells is not mutated at codon 185 [20]. Moreover, the resistance profile regarding the three substrates vincristine, daunomycin and rhodamine-123 is in general agreement with the PGP mediated phenotype of multidrug resistance.

From the chemosensitisers chosen for this study, cyclosporin A, SDZ PSC-833 and dexniguldipine have already entered clinical trials [21–23], and the potent compound GF 120918 is expected to do so in the near future. B9203-016 is a new experimental compound developed for this indication. To characterise the chemosensitising potency of these compounds, dose–response curves were generated in drug accumulation studies, and the pEC_{50}-values were used as a measure of potency. Our data showed that inhibition of PGP by each chemosensitiser was very similar for the three transport substrates rhodamine-123, vincristine and daunomycin. Although the pEC_{50}-values were not identical for a given chemosensitiser in combination with the three different substrates, the rank order of potency was independent of the measured transport substrate. Highest potency was seen with the acridone carboxamide derivative GF 120918 in all three transport test systems and also in the augmentation of vincristine cytotoxicity, identifying GF 120918 as one of the most potent chemosensitising compounds reported in the literature. High potency of GF 120918 has also been reported by others [13]. In their work, a half-maximal increase in [^{3}H]daunomycin uptake was achieved at a concentration of approximately 40 nmol/l (pEC_{50} = 7.4), which concurs with our data. The new chemosensitiser B9203-016 was almost as potent as GF 120918, while dexniguldipine and SDZ PSC-833 were approximately 10-fold less effective in the transport assays. The lower potency of cyclosporin A compared with SDZ PSC-833 is in accordance with literature data [24]. Dexniguldipine has been shown to modulate [^{3}H]daunomycin accumulation in the murine multidrug resistant F4-6RADR cell line with an pEC_{50}-value of 6.15 [25], which is somewhat lower than the values reported here. This lower potency may be due to different techniques for the isolation of the cells at the end of the drug accumulation assay or to the use of cell lines of different origin (human versus mouse). The different rank order of potency found by us for the five compounds in accumulation versus vincristine cytotoxicity tests is probably due to different serum concentrations used in both assays (0% in accumulation tests and 10% in MTT-tests). This particularly seems to hold true at low chemosensitiser concentrations, that is, at 0.3 μM (Figure 3).

It is unclear how the broad substrate specificity of PGP is achieved at the molecular level of the protein. Related to this issue is the question of whether different binding sites with high specificity for each class of compounds exist for the various drugs transported by PGP (i.e. one binding site for vinca alkaloids but another binding site for anthracyclines), or whether a common binding domain with broad specificity for different classes of drugs is involved in binding and transport. In the latter model, a similar modulation of transport of various cytostatics can be expected.

Different allosterically coupled binding sites for vinblastine and paclitaxel were identified in radioligand binding tests with [^{3}H]vinblastine and [^{3}H]paclitaxel as radioligands for PGP [26, 27]. Furthermore, binding sites for chemosensitisers of the dihydropyridine type are allosterically coupled to the binding sites for vinblastine and paclitaxel [26, 27]. A model of different binding sites for cytostatics could implicate a differential modulation of various cytostatics by a certain chemosensitiser. This would have important clinical consequences, as each chemosensitiser would have to be tested in combination with various cytostatic drugs. Alternatively, the widespread accepted PGP model of the hydrophobic 'vacuum cleaner' [28] is more compatible with a single, common hydrophobic drug-binding domain at which all substrates are bound and transported. For the latter model of a single binding domain for all cytostatics, a similar modulation of transport of different drugs could be expected and this is what we found. Although the question of a single or multiple binding sites cannot be answered by the above experiments, our data showing similar modulation of rhodamine-123, vincristine and daunomycin accumulation and transport would be more consistent with one binding domain model with broad substrate specificity, at least for the vinca-alkaloids, anthracyclines and the fluorescent dye rhodamine-123.

1. Endicott JA, Ling V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu Rev Biochem* 1989, **58**, 137–171.
2. Gottesman MM. How cancer cells evade chemotherapy: sixteenth Richard and Hinda Rosenthal Foundation award lecture. *Cancer Res* 1993, **53**, 747–754.
3. Cordon-Cardo C, O'Brian JP. The multidrug resistance phenotype in human cancer. *Important Adv Oncol* 1991, 19–38.
4. Doige CA, Sharom FJ. Transport properties of P-glycoprotein in plasma membrane vesicles from multidrug-resistant Chinese hamster ovary cells. *Biochim Biophys Acta* 1992, **1109**, 161–171.
5. Coley HM, Twentyman PR, Workman P. The efflux of anthracy-

clines in multidrug-resistant cell lines. *Biochem Pharmacol* 1993, **46**, 1317–1326.
6. Ludescher C, Hilbe W, Eisterer W, *et al.* Activity of P-glycoprotein in B-cell chronic lymphatic leukemia determined by a flow cytometric assay. *J Natl Cancer Inst* 1993, **85**, 1751–1758.
7. Beck WT. Modulators of P-glycoprotein-associated multidrug resistance. In Ozol RF, ed. *Molecular and Clinical Advances in Anticancer Drug Resistance*. Boston, Kluwer, 1991, 151–170.
8. Ford JM, Hait WN. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* 1990, **42**, 155–199.
9. Keller RP, Altermatt HJ, Nooter K, *et al.* SDZ PSC 833, a non-immunosuppressive cyclosporin: its potency in overcoming P-glycoprotein-mediated multidrug resistance of murine leukemia. *Int J Cancer* 1992, **50**, 593–597.
10. Hofmann J, Wolf A, Spitaler M, *et al.* Reversal of multidrug resistance by B859-35, a metabolite of B859-35, niguldipine, verapamil and nitrendipine. *J Cancer Res Oncol* 1992, **118**, 361–366.
11. Boer R, Haas S, Schödl A. Influence of dexniguldipine–HCl on rhodamine-123 accumulation in a multidrug resistant leukemia cell line: comparison with other chemosensitisers. *Eur J Cancer* 1994, **30A**, 1117–1123.
12. Leonce S, Pierre A, Anstett M, *et al.* Effects of the new triazinoaminopiperidine derivative on adriamycin accumulation and retention in cells displaying P-glycoprotein-mediated multidrug resistance. *Biochem Pharmacol* 1992, **44**, 1707–1715.
13. Hyafil F, Vergely C, duVignaud P, Grand-Perret T. *In vitro* and *in vivo* reversal of multidrug resistance by GF 120918, an acridonecarboxamide derivative. *Cancer Res* 1993, **53**, 4595–4602.
14. Kimmig A, Gekeler V, Neumann M, *et al.* Susceptibility of multidrug resistant human leukemia cell lines to human interleukin 2-activated killer cells. *Cancer Res* 1990, **50**, 6793–6799.
15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983, **65**, 55–63.
16. Gekeler V, Boer R, Ise W, Sanders KH, Schächtele C, Beck J. The specific bisindolylmaleimide PKC-inhibitor GF 109203X efficiently modulated MRP-associated multiple drug resistance. *Biochem Biophys Res Commun* 1995, **206**, 119–126.
17. Böhme M, Büchler M, Müller M, Keppler D. Differential inhibition by cyclosporins of primary-active ATP-dependent transporters in the hepatocyte canalicular membrane. *FEBS Lett* 1993, **333**, 193–196.
18. Barrand MA, Rhodes T, Center MS, Twentyman PR. Chemosensitisation and drug accumulation effects of cyclosporin A, PSC-833 and verapamil in human MDR large cell lung cancer cells expressing a 190k membrane protein distinct from P-glycoprotein. *Eur J Cancer* 1993, **29A**, 408–415.
19. Beck J, Handgretinger R, Dopfer R, Klingebiel T, Niethammer D, Gekeler V. Expression of mdr1, mrp, topoisomerase II/β, and cyclin A in primary or relapsed states of acute lymphoblastic leukaemias. *Br J Haematol* 1995, **89**, 356–363.
20. Gekeler V, Weger S, Probst H. MDR1/P-glycoprotein gene segments analyzed from various human leukemic cell lines exhibiting different multidrug resistance profiles. *Biochem Biophys Res Commun* 1990, **169**, 796–802.
21. Sonneveld P, Marie JP, Lokhorst HM, Nooter K, Schoester M. Clinical modulation of multidrug resistance in VAD-refractory multiple myeloma: studies with cyclosporin and SDZ PSC 833. *Anti-Cancer Drugs* 1994, **5**, 72.
22. 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**, 1652–1660.
23. Thaler J, Reiter WW, Ludescher C, *et al.* Modulation of multidrug resistance (MDR1) by dexniguldipine–HCl in combination with VAD or VECD in patients with refractory myeloma. *Annals Oncol* 1993, **67**(Suppl.), A125.
24. Boesch D, Gaveriaux C, Jachez B, Pourtier-Mazanedo 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**, 4226–4233.
25. Reymann A, Looft G, Woermann C, Dietel M, Erttmann R. Reversal of multidrug resistance in Friend leukemia cells by dexniguldipine–HCl. *Cancer Chemother Pharmacol* 1993, **32**, 25–30.
26. Ferry DR, Russel MA, Cullen MH. P-glycoprotein possesses a 1,4-dihydropyridine-selective drug acceptor site which is allosterically coupled to a vinca alkaloid-selective binding site. *Biochem Biophys Res Commun* 1992, **188**, 440–445.
27. Ferry DR, Russel MA, Kerr DJ. [^{3}H]Taxol binds to a drug acceptor site which is allosterically coupled to the vinblastine-selective site of P-glycoprotein (P-gp). *Proc Am Assoc Cancer Res* 1994, **35**, 349.
28. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* **62**, 385–427.

Acknowledgement—The skilful technical assistance of M. Giel, M. Heuser and M. Schwarz is gratefully acknowledged.