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STEREOISOMERS OF CALCIUM ANTAGONISTS WHICH DIFFER MARKEDLY IN THEIR POTENCIES AS CALCIUM BLOCKERS ARE EQUALLY EFFECTIVE IN MODULATING DRUG TRANSPORT BY P-GLYCOPROTEIN*

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Abstract—The (−)-isomer of verapamil is 10-fold more potent as a calcium antagonist than the (+)-isomer. However, both enantiomers are equally effective in increasing cellular accumulation of anticancer drugs [Gruber *et al.*, *Int J Cancer* **41**: 224–226, 1988]. In addition to verapamil, there exists a wide variety of stereoisomers with phenylalkylamines and dihydropyridine structures which markedly differ in their potency as calcium antagonists. We have tested these drugs for their ability to increase intracellular accumulation of [^{3}H]vinblastine ([^{3}H]VBL) in a doxorubicin-resistant cell line (F4-6RADR) derived from the Friend mouse leukemia cell line (F4-6P) and in COS-7 monkey kidney cells. Both cell types express substantial amounts of multidrug resistance gene 1 mRNA and P-glycoprotein as revealed by RNA and immuno blot analysis. The enantiomers with phenylalkylamine structures [(±)-verapamil; (±)-devapamil; (±)-emopamil)] and with dihydropyridine structures [(±)-isradipine; (±)-nimodipine; (±)-felodipine; (±)-nitrendipine; (±)-niguldipine] increased [^{3}H]VBL accumulation in both cell lines at micromolar concentrations. Although the stereoisomers of these drugs differ markedly in their potency as calcium channel blockers they were about equally effective in increasing VBL levels in the cells. There was no substantial difference in the potencies of the phenylalkylamine drugs in affecting cellular [^{3}H]VBL transport. Major potency differences, however, were observed in the dihydropyridine drug series with the niguldipine isomers as the most effective drugs. Moreover, the niguldipine enantiomers were equally as effective in reversing VBL resistance in F4-6RADR cells as were the verapamil enantiomers. Since (−)-niguldipine (B859-35) displays a 45-fold lower affinity for calcium channel binding sites than (+)-niguldipine, but is equally potent in inhibiting drug transport by P-glycoprotein and in reversing drug resistance, it may be, in addition to (+)-verapamil, another useful candidate drug for the treatment of multidrug resistance in cancer patients.

In addition to their well-characterized interaction with the α_1-subunit of the voltage-dependent L-type calcium channel, a variety of calcium antagonists, such as verapamil, modulate the P-glycoprotein-induced extrusion of cytotoxic substances at micromolar concentrations [1]. These drugs are, therefore, of potential therapeutic use for enhancing the cytotoxicity of chemotherapeutic agents. A limiting factor, however, is the pronounced cardiovascular effects of the calcium channel antagonists which occur at the high plasma concentrations required to block efficiently P-glycoprotein transport. *In vitro* and *in vivo* studies, however, have shown that the (+ or *R*)-isomer of verapamil is 10-fold less potent as a calcium antagonist than the (− or *S*)-isomer [2, 3]. On the other hand, both enantiomers are about equally effective in increasing the cellular accumulation of anticancer drugs [4–6]. (+)-Verapamil, therefore, should be superior to racemic verapamil in enhancing the cytotoxicity of anticancer drugs.

In addition to verapamil, there exists a wide variety of stereoisomers with phenylalkylamine and dihydropyridine structures which differ markedly in their potency as calcium antagonists [7–9]. We have tested these drugs for their ability to increase intracellular accumulation of [^{3}H]vinblastine ([^{3}H]-VBL§) in doxorubicin-resistant murine leukemia cells (F4-6RADR) and in COS-7 monkey kidney cells. In addition, some of the enantiomers will be shown to reverse drug resistance in F4-6RADR cells.

MATERIALS AND METHODS

Materials. The (+)- and (−)-enantiomers of verapamil, devapamil and emopamil as well as of gallopamil were kindly provided by Dr Traugott (Knoll AG, Ludwigshafen, F.R.G.); (+)- and (−)-nimodipine and BayK 8644 were generous gifts from Drs Glaser and Traber (Tropon Werke, Köln, F.R.G.); (+)-niguldipine (B859-34), (−)-niguldipine (B859-35), the common metabolite of (+)- and (−)-niguldipine (3-3-(4,4-diphenyl-1-piperidinyl)-propyl-5-methyl-4-(3-nitrophenyl)-2,6-dimethyl-pyridine-3,5-dicarboxylate), the stereoisomers B869-60 ((−)-3-3-(4,4-diphenyl-1-piperidinyl)-propyl-5-methyl-4(*S*)-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-

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§ Abbreviations: *MDR*1, multidrug resistance gene 1; VBL, vinblastine; DMSO, dimethyl sulfoxide.

pyridine-3,5-dicarboxylate-hydrochloride) and B869-61 ((+)-3-3-(4,4-diphenyl-1-piperidinyl)-propyl 5-methyl-4(*R*)-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-pyridine-3,5-dicarboxylate-hydrochloride) as well as the (+)- or (−)-enantiomers of felodipine, isradipine and nitrendipine were gifts from Dr Sanders (Byk Gulden-Lomberg GmbH, Konstanz, F.R.G.). The optical purity of the enantiomers was greater than 99%. The drugs were dissolved and diluted in dimethyl sulfoxide (DMSO). The final DMSO concentration in the cell incubation media was 0.1% (v/v). [^{3}H]VBL sulfate (sp. act. 11.7 Ci/mmol) was purchased from Amersham-Buchler (Braunschweig, F.R.G.); [^{14}C](−)-niguldipine (B859-35) (sp. act. 50 mCi/mmol) was kindly provided by Dr Zech (Byk Gulden-Lomberg GmbH).

Cells. Drug-resistant and -sensitive murine leukemia cells were obtained from Dr Erttmann (Hamburg, F.R.G.). These doxorubicin-resistant cells (F4-6RADR) represent a stable subline which has been established from the Friend leukemia cell line (F4-6P) [10]. The cells exhibit a 50-fold resistance to doxorubicin [11]. COS-1, COS-7 [12] and CV1 monkey kidney cells were gifts from Dr Renkawitz (Martinsried, F.R.G.). The cells were propagated in Dulbecco's minimal essential medium supplemented with 10% fetal calf serum and antibiotics.

The cells were plated in cluster wells (6 well; Costar) and grown to 60–80% confluency in 1 mL medium. On the day of the experiment, the reaction was started by adding varying concentrations of calcium blocker to the 1 mL incubation medium. The final concentration of DMSO was 0.1%. After 20 min preincubation, [^{3}H]VBL (2.5 nmol/L) was added (final concentration of ethanol 0.1%). The reaction was stopped by removing the medium rapidly. Thereafter, the cells were detached from the cells with 1 mL ice-cold Ringer solution and the cell suspensions filtered rapidly through glass fiber filters (GFB Whatman). The filters were washed twice with 5 mL ice-cold Ringer solution and added to scintillation vials. After adding scintillation fluid the radioactivity on the filters was counted in a β-counter with 40% efficiency.

Cytotoxicity test. F4-6P or F4-6RADR cells (3×10^4) were plated with Dulbecco's minimal essential medium containing 10% calf serum in the absence or presence of increasing doses of VBL and/or of calcium antagonists. After 84 hr the cells were counted under a microscope.

Immunoblotting. The isolation of plasma membranes from the cells was performed according to a technique described by Volm *et al.* [13]. The cells (COS-1, COS-7, CV-1; F4-6P, F4-6RADR) were homogenized in hypotonic Tris-buffer (15 mM Tris–HCl, pH 8.0, 1 M $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride) using a polytron homogenizer. The homogenates were centrifuged at 800 *g* for 10 min to pellet nuclei, at 4000 *g* for 10 min to pellet mitochondria and finally at 100,000 *g* for 30 min to obtain the membrane fraction. SDS–PAGE was performed according to the technique described by Fairbanks *et al.* [14]. Protein concentrations of the extracts were determined by the method provided by BioRad. Aliquots (80 μg) of membrane proteins was loaded onto the gel. After electrophoresis electroblotting was performed according to the procedure of Towbin *et al.* [15]. After transfer, the nylon filters (Nytran, Schleicher-Schuell) were incubated in blocking solution (3% w/v bovine serum albumin in phosphate-buffered saline, 15 mM sodium azide) over night at 37°. The filters were then incubated at 37° for 30 min with monoclonal antibody C219 (Glycocheck, Centrocor, Malven, U.S.A.) at a dilution of 1 μg/mL in TBST buffer (10 mM Tris–HCl, pH 8, 150 mM NaCl, 0.05% Tween 20). After washing the membranes in TBST three times for 5 min, the nylon filters were incubated for 30 min at 37° with the second antibody (alkaline phosphatase-conjugated anti-mouse immunoglobulin; dilution 1:1000 in TBST; Dakopatts, Glostrup, Denmark). After washing the filters were incubated in alkaline phosphate buffer (100 mM Tris–HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) containing nitroblue tetrazolium.

RNA blotting. RNA was isolated from the cells by a lithium chloride technique [16]. RNA samples were denatured with glyoxal and 5-μg aliquots were electrophoresed on a 1.2% agarose gel and transferred to nylon filters. The filters were hybridized at 60° to a radiolabelled human multidrug resistance gene 1 (*MDR*1) cRNA probe. The plasmid 5 A ([17], a gift from Drs Pastan and Gottesman, Bethesda, U.S.A.), was used to make the cRNA probe with Sp 6 RNA polymerase. After hybridization the filters were washed, dried and exposed to X-ray film at −70°.

Calculations. The potencies, i.e. the concentrations of the drugs which cause a half-maximal increase in cellular [^{3}H]VBL concentration (apparent EC_{50}), were calculated from dose–response curves using six different concentrations of calcium channel blocker ranging from 0.3 to 30 μM. Maximal concentrations are those of [^{3}H]VBL in drug-sensitive F4-6P cells or in COS-7 cells in the presence of 30 μM verapamil. The values were expressed in per cent of the maximal effects. The values were subjected to log probit analysis and the EC_{50} data computed from regression lines. At least three independent dose–response curves were calculated for each individual drug and cell type.

RESULTS

Doxorubicin-resistant mouse leukemia cells (F4-6RADR) express high levels of *MDR*1 mRNA (Fig. 1 left, lane 4) and P-glycoprotein (Fig. 1 right, lane 4) in contrast to the drug-sensitive parent cell line F4-6P (Fig. 1, lanes 5). Significant concentrations of *MDR*1-like mRNA were also seen in cell lines derived from monkey kidney cells, such as CV-1, COS-1 and COS-7 (Fig. 1 left, lanes 1–3). These cells also express P-glycoprotein as revealed by immunoblot analysis (Fig. 1 right, lanes 1–3).

F4-6RADR cells accumulated very small amounts of [^{3}H]VBL as compared with the drug-sensitive F4-6P cells, when incubated with the anticancer drug *in vitro* for 1 hr (Fig. 2). However, the drug-resistant F4-6RADR cells showed an increased content of [^{3}H]VBL, when co-incubated with calcium channel antagonists at doses between 0.3 and 30 μM. The

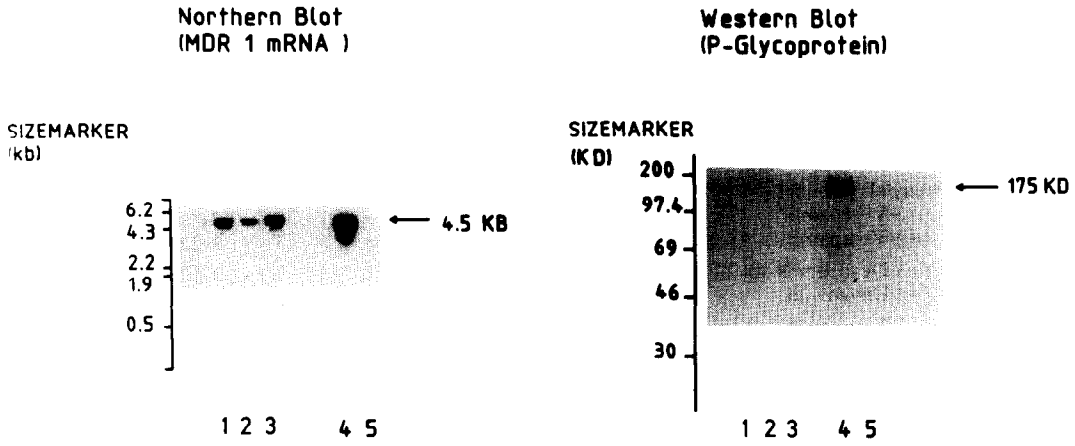

Fig. 1. Doxorubicin-resistant leukemia cells (F4-6RADR) and COS-7 kidney cells express *MDR*1 mRNA and P-glycoprotein. Left: 5-μg aliquots of total RNA were extracted from cells and subjected to agarose electrophoresis and RNA blot analysis as described in Materials and Methods. λDNA/*Hind* III fragments served as size markers. Right: 80-μg aliquots of cell membrane protein were subjected to polyacrylamide electrophoresis and immunoblot analysis as described in Materials and Methods. Rainbow markers (Amersham) served as size standards. Lane 1 = COS-1; lane 2 = COS-7; lane 3 = CV-1; lane 4 = F4-6RADR; lane 5 = F4-6P.

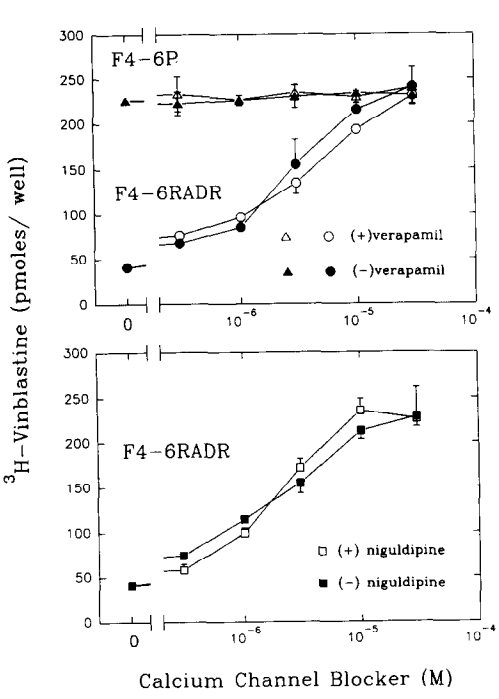

Fig. 2. Dose-dependent effect of the stereoisomers of verapamil and niguldipine on the accumulation of [^{3}H]-VBL in doxorubicin-resistant (F4-6RADR) and -sensitive (F4-6P) cells. Cells were incubated with 2.5 nM [^{3}H]VBL in the presence of (+)-verapamil, (−)-verapamil, (+)-niguldipine or (−)-niguldipine. After 60 min cellular [^{3}H]-VBL was measured as described in Materials and Methods. Values are means ± SEM of 3–4 wells.

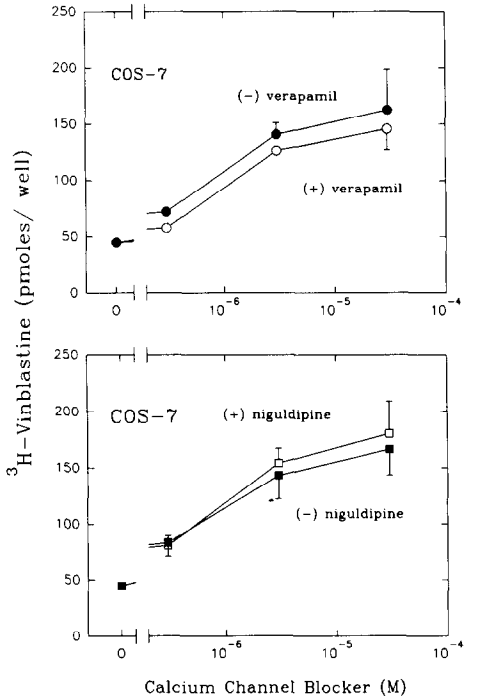

Fig. 3. Effect of verapamil and niguldipine enantiomers on VBL accumulation in COS-7 monkey kidney cells. Cells were incubated with 2.5 nM [^{3}H]VBL in the presence of (+)-, (−)-verapamil or (+)-, (−)-niguldipine. After 60 min cellular content of [^{3}H]VBL was measured as described in Materials and Methods. Values are means ± SEM of four wells.

Table 1. Relative potencies of various calcium antagonists for inhibition of cellular VBL accumulation

Drug	Doxorubicin-resistant mouse leukemia cells (F4-6RADR)	Monkey kidney cells (COS-7)
	EC_{50} (μM)	EC_{50} (μM)
Phenylalkylamines		
(−)-Verapamil	2.9 (2.3–3.5)	2.4 (2.0–3.0)
(+)-Verapamil	2.6 (2.2–3.0)	1.6 (1.3–2.0)
(+)-Devapamil	1.0 (0.8–1.3)	1.3 (1.0–1.8)
(−)-Devapamil	2.1 (1.4–2.7)	3.7 (2.0–4.5)
(−)-Emopamil	3.0 (2.7–3.5)	3.7 (3.0–4.7)
(+)-Emopamil	2.4 (2.2–2.6)	3.8 (3.1–4.4)
Gallopamil	2.5 (2.0–3.9)	2.4 (1.9–2.8)
Dihydropyridines		
(+)-Niguldipine	1.2 (0.9–1.4)	1.1 (0.8–1.4)
(−)-Niguldipine (B859-35)	1.3 (0.9–1.5)	1.1 (0.7–1.5)
Common metabolite	0.7 (0.6–0.9)	0.6 (0.3–0.9)
(+)-Isradipine	9.2 (8.4–10.1)	6.9 (6.2–7.4)
(−)-Isradipine	4.7 (4.0–5.8)	4.0 (3.5–5.0)
(−)-Nitrendipine	9.4 (8.4–11.0)	8.6 (7.0–9.5)
(+)-Nitrendipine	10.3 (9.1–12.1)	5.7 (5.2–6.5)
(+)-Nimodipine	12.6 (10.1–13.0)	5.2 (4.3–6.1)
(−)-Nimodipine	15.8 (12.9–17.0)	6.7 (5.0–7.5)
(−)-Felodipine	8.6 (8.0–8.9)	19.2 (17–22)
(+)-Felodipine	6.3 (5.9–7.0)	19.6 (17–23)
B869-60	58.0 (52–65)	67.8 (59–77)
B869-61	50.0 (45–57)	51.3 (42–63)
Nifedipine	73.0 (65–80)	74.0 (69–80)
Bay K 8644	79.0 (68–90)	100.0 (88–111)

Apparent EC_{50} values are the concentrations of the drugs which cause a half-maximal increase in cellular [^{3}H]VBL accumulation (see Materials and Methods). Values represent mean values of 3–4 dose–response curves. Maximum and minimum values are given in brackets.

stereoisomers (+)- and (−)-verapamil and (+)- and (−)-niguldipine are about equally effective in increasing cellular concentrations of [^{3}H]VBL. The calcium channel drugs also increased the accumulation of [^{3}H]VBL in COS-7 cells which contain measurable levels of *MDR*1 mRNA or P-glycoprotein (Figs 1 and 3). A half maximal increase in [^{3}H]VBL concentration occurred at verapamil or niguldipine concentrations of between 1 and 3 μM. There was no major difference in the potencies of the stereoisomers of these drugs in augmenting [^{3}H]VBL accumulation in F4-6RADR and COS-7 cells.

Table 1 compares the potencies of a wide variety of calcium channel-modifying drugs in augmenting cellular accumulation of [^{3}H]VBL in F4-6RADR cells and in COS-7 cells. The enantiomers of a respective calcium channel blocker were as effective in increasing the accumulation of [^{3}H]VBL in F4-6RADR cells as in COS-7 cells. The phenylalkylamine derivatives (the enantiomers of verapamil, devapamil, emopamil, as well as gallopamil) were equally effective in enhancing [^{3}H]VBL accumulation in the two cell types. In contrast, major potency differences were observed amongst the dihydropyridine derivatives. The enantiomers of niguldipine and particularly their common metabolite (see Materials and Methods) were the most potent compounds in increasing the cellular accumulation of [^{3}H]VBL. On the other hand, the calcium channel antagonist nifedipine and the calcium agonist BayK 8644 exhibited an about 50–100-fold lower potency than the niguldipine isomers or the phenylalkylamine derivatives in increasing the cellular levels of the *Vinca* alkaloid. The apparent potencies of the dihydropyridine derivatives are similar in F4-6RADR cells and in COS-7 cells.

Table 2 compares the potencies of enantiomers of various calcium channel antagonists in increasing [^{3}H]VBL accumulation with their abilities to inhibit the K^+-induced contraction of the rat aortic strips *in vitro* [18] or to inhibit the binding of [^{3}H]isradipine to calcium channel binding sites in guinea-pig skeletal muscle membranes [9] (Boer, unpublished data). In contrast to their about equal potency in augmenting [^{3}H]VBL accumulation in P-glycoprotein-expressing cells, the enantiomers differ markedly in the activity on or binding affinity for calcium channel sites. The largest potency differences were observed for

Table 2. Activities of phenylalkylamines and dihydropyridine enantiomers on calcium channels and on [^{3}H]VBL accumulation

Phenylalkylamines	Calcium antagonism on rat aortic stripes		Enhancement of VBL accumulation in F4-6RADR cells	
	EC_{50} (nM)	Potency ratio	EC_{50} (μM)	Potency ratio
(−)-Verapamil	24*	9.2	2.9	0.9
(+)-Verapamil	220*		2.6	
(−)-Devapamil	2*	195	1.0	2.1
(+)-Devapamil	390*		2.1	
(−)-Emopamil	270†	0.8	3.0	0.8
(+)-Emopamil	220†		2.4	
Dihydropyridines	**[^{3}H]Isradipine binding (guinea-pig skeletal muscle)**		**Enhancement of VBL accumulation in F4-6RADR cells**	
	K_i (nM)	Affinity ratio	EC_{50} (μM)	Potency ratio
(+)-Niguldipine	0.18‡	452	1.2	1.1
(−)-Niguldipine	8.1‡		1.3	
(−)-Nitrendipine	1.3‡	10	9.4	1.1
(+)-Nitrendipine	13‡		10.3	
(−)-Felodipine	0.19‡	12.6	8.6	0.7
(+)-Felodipine	2.4‡		4.7	
(+)-Isradipine	0.68§	125	9.2	0.5
(−)-Isradipine	85§		4.7	
B869-60	3.8§	4.5	58	0.9
B869-61	17.4§		50	

Values from calcium antagonism on rat aortic stripes are from *Ref. 44 and from †Ref. 18; values for inhibition of [^{3}H]isradipine binding are from ‡Ref. 9 and §Boer *et al.*, unpublished data. Values for [^{3}H]VBL accumulation are from Table 1. Means of 3–6 independent experiments are given.

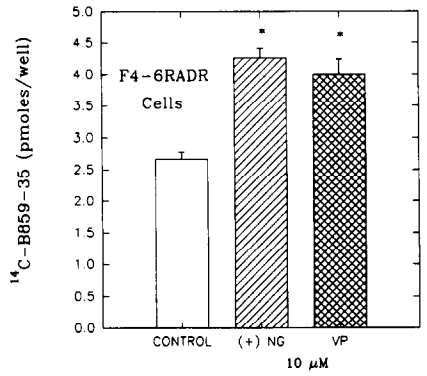

Fig. 4. Increase in the accumulation of [^{14}C]B859-35 [(−)-niguldipine] in doxorubicin-resistant F4-6RADR leukemia cells by (+)-niguldipine and verapamil. Cells were incubated with 20 nM [^{14}C]B859-35 in the absence (filled bar) or presence of (+)-niguldipine (NG; open bar) or verapamil (VP; cross-hatched bar). Values are means ± SEM of six wells. Asterisks indicate significant differences ($P < 0.005$) as revealed by Student's *t*-test.

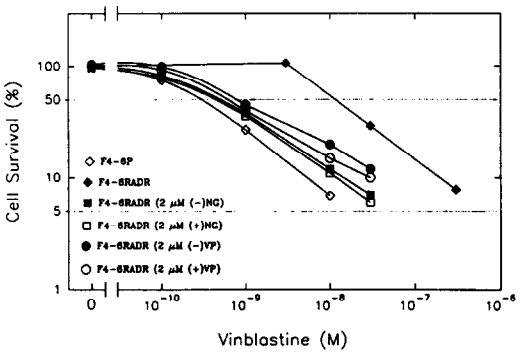

Fig. 5. Cytotoxic effects of VBL on doxorubicin-resistant (F4-6RADR) and -sensitive (F4-6P) cells in the absence or presence of verapamil (VP) or niguldipine (NG) enantiomers. The cells were grown with increasing doses of VBL in the presence or absence of 2 μM (+)-, (−)-verapamil or (+)-, (−)-niguldipine. After 84 hr the cells were counted. Values are means of four wells. EC_{50} values of VBL: F4-6P = 3.2×10^{-10} M; F4-6RADR = 1×10^{-8} M; F4-6RADR plus 2 μM (+)-niguldipine = 5.5×10^{-10} M; F4-6RADR plus 2 μM (−)-niguldipine = 5.9×10^{-10} M; F4-6RADR plus 2μM (−)-verapamil = 6.8×10^{-10} M; F4-6RADR plus 2 μM (+)-verapamil = 7.5×10^{-10} M.

devapamil (195-fold), isradipine (125-fold) and niguldipine enantiomers (45-fold).

Figure 4 illustrates that the accumulation of [^{14}C]-B859-35 ((−)-niguldipine) in cells can be increased by co-incubation with high concentrations of unlabeled (+)-niguldipine and/or racemic verapamil indicating that the calcium antagonists compete mutually with each other for transport by P-glycoprotein.

Figure 5 shows that VBL is about 30 times more cytotoxic for F4-6P cells than for doxorubicin-

resistant F4-6RADR cells. Co-incubation with verapamil or niguldipine isomers at 2 μM concentration increased the cytotoxicity of VBL for the F4-6RADR cells by about the same extent (13–18-fold). At these concentrations the calcium channel drugs did not have any effect on VBL toxicity toward F4-6P cells and did not exert their own cytotoxic effects (data not shown).

DISCUSSION

The experiments reported here show that the stereoisomers of a wide variety of calcium antagonists differ markedly in their potency as calcium channel blockers, but are equally effective in increasing the accumulation of [^{3}H]VBL in two P-glycoprotein-expressing cell lines. These results extend and confirm earlier findings of Gruber *et al.* [4] who reported that racemic verapamil, as well as the (−)- and the (+)-isomers of verapamil, increased cellular vincristine accumulation in human *MDR* expressing leukemia cells to the same extent. Similar findings were also obtained by Plumb *et al.* [5] and Keilhauer *et al.* [6]. These groups also showed that the verapamil enantiomers increased the sensitivity to cytotoxic drugs in *MDR* expressing cells with the same potency. In contrast, a recent paper reported that (−)-verapamil is about 4-fold more active than (+)-verapamil in modifying multidrug resistance in human KB-8-5 cells [19]. The reason for this discrepancy is not known. A stereoselective modification of multidrug resistance and of doxorubicin accumulation in doxorubicin-resistant MCF-7 cells was recently reported for various thioxanthene derivatives [20]. On these cells *trans*-flupenthixol was more potent than *cis*-flupenthixol.

In our experiments, the potencies of the phenylalkylamine derivatives ((+)- and (−)-verapamil; (−)- and (+)-devapamil; (−)- and (+)-emopamil and gallopamil) in increasing [^{3}H]VBL accumulation in F4-6RADR and COS-7 cells were similar. Marked potency differences, however, were observed for the dihydropyridine derivatives. Whereas the niguldipine enantiomers exhibited a relatively high potency, nifedipine or the calcium channel agonist BayK 8644 were much less potent in increasing cellular [^{3}H]-VBL accumulation. Nifedipine has been shown recently to be less effective than verapamil in inhibiting ATP-dependent VBL transport by vesicles of drug-resistant cells [21]. The reason for the different potencies amongst the dihydropyridine drug series is not completely clear. The niguldipine enantiomers exhibit, in addition to their calcium channel blocking activity, potent α_1 adrenoceptor antagonistic effects [7]. Interestingly, α_1 adrenoceptor antagonists bind to P-glycoprotein as revealed in photolabeling experiments using [^{125}I] iodoarylazidoprazosin [22, 23]. It is thus possible that the niguldipine drugs can interact with P-glycoprotein via its calcium antagonistic and its α_1 adrenoceptor antagonistic portion of the molecule. In addition, the niguldipine enantiomers and, in particular, their common metabolite, are extremely lipophilic compounds ($\log P = 4.3$; octanol/H_2O at pH 7.4 for the niguldipine enantiomers [24]). It is well known that lipophilic drugs interfere with the cellular transport of anticancer drugs by P-glycoprotein (e.g. Ref. 25) and P-glycoprotein has been proposed to function as a "vacuum cleaner" of the cell or as flippase for hydrophobic compounds ([26]; M. Gottesman, AACR Conference, Banff, Canada, 1991, unpublished).

There is additional evidence that the mechanism by which the various calcium channel blockers interfere with the accumulation of [^{3}H]VBL involves P-glycoprotein. Thus, verapamil has been shown to displace [^{3}H]azidopine from its binding to Pglycoprotein [27]. Moreover, the transepithelial transport of verapamil in Madin-Darby canine kidney cells (MDCK) can be blocked by VBL. This provides further evidence that verapamil is a competitive substrate for transport by P-glycoprotein [28]. The niguldipine enantiomers also appear to be exported by P-glycoprotein, since the accumulation of [^{14}C] B859-35 ((−)-niguldipine) in F4-6RADR cells is increased by (+)-niguldipine and by racemic verapamil (Fig. 4). On the other hand, there may be other and/or additional mechanisms whereby calcium channel drugs influence the transport function of P-glycoprotein. For instance, some calcium channel blockers have been shown to be potent inhibitors of calmodulin-dependent cAMP phosphodiesterases. B859-35 and its opposite enantiomer (+)-niguldipine exhibit a similarly high potency as calmodulin inhibitors and it has been suggested that the calmodulin antagonism may be responsible for the inhibitory effect of B859-35 on cell proliferation *in vitro* and *in vivo* [29, 30]. On the other hand, the calmodulin antagonism does not appear to have a general role in modifying the transport function of P-glycoprotein, since (+)-nimodipine is by order of magnitude more potent than (−)-nimodipine as a calmodulin antagonist, whereas they exhibit a similar potency in influencing P-glycoprotein transport [31]. Moreover, in a larger study no correlation was found between anti-calmodulin activity and antagonism of multidrug resistance for a great number of phenothiazine drugs [32].

The relative potencies of the majority of the calcium channel blockers in blocking the export of anticancer drugs is low (apparent EC_{50} at micromolar concentrations) as compared to their calcium blocking activity which occurs at nanomolar concentrations. Thus, micromolar plasma levels have to be achieved in clinical studies for enhancing the cytotoxicity of various chemotherapeutic agents. One limiting factor proved to be the inherent cardiovascular activities of the calcium channel drugs (hypotension and/or prolonged atrioventricular conduction time) as observed in first clinical studies in which verapamil was used for reversing drug resistance (e.g. Refs 33 and 34). Although a recent study demonstrated that in the case of malignant lymphoma chemotherapy, maximally tolerated doses of verapamil resulted in a high response rate in patients carefully selected for clinical drug resistance [35], a further improvement in reversing drug resistance by verapamil may be the use of the (+)-isomer which is less cardiovascularly active but exhibits a similar potency to racemic verapamil in blocking the cellular export of anticancer drugs. In

fact, high doses of (+)-verapamil (about 1000 mg/day) are relatively well tolerated in humans and produce plasma concentrations of about 2 μg/mL (about 4 μmol/L), which should be effective for adjuvant cancer therapy [36].

Based on the potency ratios of the enantiomers between their activity at the calcium channel and their activity in modifying P-glycoprotein transport we would suggest that (+)-devapamil, which is almost 200-fold less potent as a calcium antagonist than (−)-devapamil but exhibits an about equal potency for P-glycoprotein transport, might be a useful drug for reversing drug resistance. However, a recent study showed that devapamil only partially reverses multidrug resistance in human KB cell lines [37].

(−)-Niguldipine (B859-35) exhibits an about 45-fold lower potency in inhibiting binding to calcium channel sites than (+)-niguldipine. In addition, B859-35 is one of the most potent drugs in increasing cellular accumulation of [^{3}H]VBL. Moreover, our experiments show that B859-35 is similarly effective in reversing drug resistance in F4-6RADR cells as its stereoisomer (+)-niguldipine and the verapamil enantiomers. Furthermore, B859-35 has also been shown to effectively reverse resistance in various vincristine and doxorubicin-resistant cell lines [38]. Moreover, it appears to be less toxic than verapamil in drug-sensitive cells [39, 40]. B859-35 might therefore be a good candidate drug for enhancing the cytotoxicity of chemotherapeutic drugs in clinical studies *in vivo*. An interesting finding was that COS-7 cells which derive from monkey kidney can be used as a model for studying P-glycoprotein transport. The cells express P-glycoprotein and hybridize with a human *MDR*1 probe. Moreover, the relative potencies of the various calcium channel drugs in increasing [^{3}H]VBL accumulation are similar to that found in F4-6RADR murine leukemia cells indicating that binding and transport characteristics of P-glycoprotein in the two cell types are similar. It is noteworthy to mention in this context that high levels of *MDR*1 mRNA were also found in human kidneys [17] and in kidney cancers [41, 42]. In addition, expression of *MDR*1 mRNA has been found in other kidney cells (MDCK; OK; LLC-PK1 [43]).

The COS-7 monkey kidney cells contain an origin-defective mutant of SV 40 [12]. This allows the effective replication of expression vectors containing an intact SV 40 origin. In a preliminary study we have transfected COS-7 cells with a *MDR*1 antisense vector and found an increased accumulation of [^{3}H] VBL indicating that the antisense vector inhibits synthesis of P-glycoprotein in these cells. In addition, the possibility to express variants of *MDR* genes in COS-7 cells should offer new possibilities of studying the function of *MDR* transporters.

In conclusion, our results show that a wide variety of stereoisomers of calcium channel antagonists which differ markedly in their potency in blocking calcium channels are equally potent in increasing [^{3}H]VBL in P-glycoprotein-expressing cells. Of the dihydropyridine drugs the niguldipine enantiomers are the most potent drugs and are as potent as the verapamil isomers in reversing drug resistance in F4-6RADR leukemia cells *in vitro*. (−)-Niguldipine (B859-35) which is 45-fold less potent than (+)-niguldipine as a calcium channel blocker may be a useful candidate drug for reversing multidrug resistance in cancer patients.

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