

# Potentialiation of doxorubicin cytotoxicity by the calcium antagonist bepridil in anthracycline-resistant and -sensitive cell lines

## A comparison with verapamil

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**Summary.** The ability of the calcium channel blocker bepridil (Bp) to potentiate doxorubicin (Dx) cytotoxicity and enhance its accumulation in anthracycline-sensitive and -resistant human ovarian carcinoma cells (A2780 and 2780<sup>AD</sup>) and Chinese hamster ovarian (CHO) cells (AUXB1 and CH<sup>R</sup>C5) was compared with that of verapamil (Vp). A continuous exposure (48–72 h) to Bp as well as Vp potentiated Dx cytotoxicity in 2780<sup>AD</sup> cells. In short-term incubations (2 h Dx and 4 h calcium channel blocker) the same effects were observed: 4  $\mu$ M Bp (4Bp) and Vp (4Vp) were equipotent, but at concentrations of 1 and 2  $\mu$ M, Vp was more active (4Vp = 4Bp > 2Vp > 2Bp > 1Vp > 1Bp). In CH<sup>R</sup>C5 cells the corresponding sequence was: 4Vp > 4Bp > 2Vp > 2Bp > 1Vp > 1Bp. At high (marginally inhibitory) concentrations, Bp and Vp reversed Dx resistance completely in CH<sup>R</sup>C5 cells and partly in 2780<sup>AD</sup> cells. No significant potentiation of Dx cytotoxicity by Bp or Vp was found in A2780 cells, but both were active in AUXB1 cells. In studies with radiolabelled Dx (2  $\mu$ M), Bp and Vp (16.5  $\mu$ M) stimulated accumulation in CH<sup>R</sup>C5 cells almost up to the level in AUXB1 cells. Dx accumulation in 2780<sup>AD</sup> cells (52 pmol/10<sup>6</sup> cells) could be stimulated to a maximum of about 90 pmol/10<sup>6</sup> cells (drug level in A2780 cells was 153 pmol/10<sup>6</sup> cells). Also, Bp- and Vp-induced stimulation of Dx accumulation was observed in AUXB1, but not in A2780 cells. Bp effected a dose-dependent inhibition of Dx efflux from preloaded 2780<sup>AD</sup> cells, but not from A2780 cells. We conclude that Bp is more effective than Vp in reversing resistance when both compounds are used in vitro at concentrations which are clinically achievable in plasma.

## Introduction

A major problem in the treatment of cancer patients is the development of resistance to the drugs used in chemotherapy [10]. One way to overcome in vitro resistance to certain anticancer drugs derived from natural products is the administration of calcium channel-blockers or calmodulin inhibitors with the anticancer drug used [4]. Following the initial studies of Tsuruo et al. with Vp in vincristine-resis-

tant mouse leukemia (P388) cells [14], many compounds with calcium channel-blocking or calmodulin-inhibiting activity have been studied to determine their effects on in vitro drug sensitivity in mouse [11, 13, 15, 17, 18] as well as human [1, 7, 12, 13, 16, 17] tumor cells. Vp is one of the compounds with the greatest in vitro effect on vincristine or Dx cytotoxicity in resistant mouse and human tumor cells [15, 16]. It has been shown that at least part of the effects of Vp can be ascribed to its ability to inhibit the efflux of the anticancer drugs used [4, 9]. Several blockers or calmodulin inhibitors have been shown to potentiate the positive effects of vincristine or Dx on the life span of P388/VCR or P388/ADR tumor-bearing mice [14, 17] as well as daunomycin-resistant Ehrlich ascites tumor-bearing mice [11]. In vitro studies that show clear effects of relatively low concentrations of Vp (about 0.5  $\mu$ M), which can actually be achieved clinically [3], have rarely been reported [7]. Therefore, it is important to search for agents which can potentiate drug sensitivity in resistant tumor cells at clinically achievable concentrations. Recently we presented preliminary data suggesting that the calcium channel blocker Bp is such a compound [8]; effective levels at plasma concentrations of 2–4  $\mu$ M have been achieved without development of toxicity during treatment of angina pectoris [2]. The effects of these concentrations of Bp on Dx sensitivity of anthracycline-resistant and -sensitive cell lines are described and compared with those of Vp. The effects observed are then related to the effects of a range of calcium channel-blocker concentrations on Dx accumulation.

## Materials and methods

**Drugs.** Bepridil monohydrochloride monohydrate,  $\beta$ -(2-methylpropoxy)-methyl]-N-phenyl-N-(phenylmethyl)-1-pyrrolidine ethanamine (ORG 5730), was obtained from Organon International B. V. (Oss, The Netherlands) as a sterile 4 mg/ml solution containing 48.4 mg/ml glucose. Verapamil and colchicine were bought from Sigma Chemical Company. Doxorubicin (Adriablastin) was obtained from Laboratoire Roger Bellon. [<sup>14</sup>C]14-Dx (53.3  $\mu$ Ci/ $\mu$ mol) was purchased from the Radiochemical Centre (Amersham, England). Concentrated drug solutions and drug dilutions were prepared in 0.9% NaCl.

**Cells and cell culture.** The Dx-sensitive human ovarian carcinoma cell line A2780 and its resistant subline 2780<sup>AD</sup>

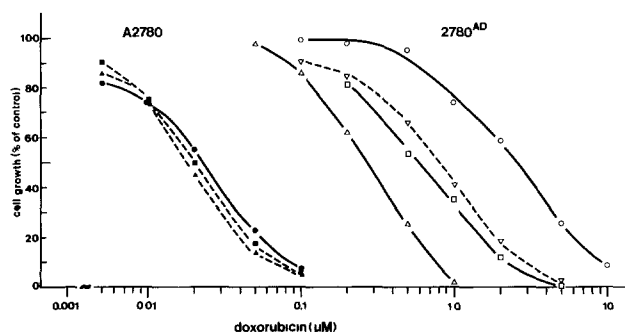
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Abbreviations used: Bp, bepridil; CHO, Chinese hamster ovarian; DMF, dose-modifying factor; Dx, doxorubicin; Vp, verapamil

were supplied by Dr. R. F. Ozols (National Cancer Institute, Bethesda MD USA). The CHO cell line AUXB1 and its colchicine-resistant subline CH<sup>R</sup>C5 were obtained from Dr. V. Ling (Ontario Cancer Institute, Ontario, Canada). Cells were maintained in plastic flasks (C. A. Greiner, Germany) in a humidified atmosphere at 5% CO<sub>2</sub>. The human cell lines were cultured in Dulbecco's modification of Eagle's medium with glutamine (DMEM, Gibco, Europe Ltd, UK) containing 20 mM HEPES and supplemented with 10% fetal bovine serum (Flow Laboratories, UK). CHO cells were maintained in MEM Alpha Medium (Gibco, Europe Ltd) with 10% fetal bovine serum. The resistant cell lines were cultured in the presence of drugs (2  $\mu$ M Dx for 2780<sup>AD</sup> and 10  $\mu$ g/ml colchicine for CH<sup>R</sup>C5). Experiments were performed after culturing in drug-free medium for 1–2 weeks. Doubling times of the cells were 14, 25, 15, and 25 h, respectively, for AUXB1, CH<sup>R</sup>C5, A2780, and 2780<sup>AD</sup>.

**Drug treatment.** Log-phase cells were harvested from flasks using trypsin/EDTA. Cells were plated in six-well tissue culture clusters (Costar, USA) (3–5.10<sup>4</sup> cells per well). Logarithmically growing cells were exposed to Dx only or to Dx plus Bp or Vp during the period needed for three cell divisions. Besides experiments with continuous exposure, an alternative scheme was used: cells were exposed to the blockers for 15 min at 37°C, after which Dx was added for 2 h. Thereafter the medium was replaced by one containing only the blocker, in which the cells were kept for 2 h at 37°C before replacement by drug-free medium. Cells were then cultured as described above. Control experiments involved exposure to one of the drugs or to neither. After culturing, the cells were trypsinized and counted with a Coulter counter (Sigmex microcell counter CC-110). Cell numbers were corrected for the number of cells present at the time of addition of the drugs (thus indicating cell growth). The effects of the blockers were expressed as DMFs: IC<sub>50</sub> Dx alone/IC<sub>50</sub> Dx + blocker.

**Drug accumulation and efflux.** Human ovarian cancer cells in log-phase were harvested, washed, and resuspended to a concentration of about 10<sup>6</sup> cells/ml in DMEM pH 7.4 without phenol red and NaHCO<sub>3</sub>, but with 20 mM HEPES. Concentrated solutions of either Bp or Vp and [<sup>14</sup>C]14-Dx were added to 500- $\mu$ l cell suspensions in 2-ml Eppendorf vials at 0°C, to give a final volume of 550  $\mu$ l and a final blocker or Dx concentration of 1–20  $\mu$ M or 2  $\mu$ M, respectively. Cells were incubated for 0–90 min at 37°C; at different times 550- $\mu$ l cell samples were rapidly cooled with ice-cold PBS and centrifuged for 0.5 min at full speed in an Eppendorf centrifuge 3200. After an additional 2-ml washing step, the cells were transferred in 400  $\mu$ l PBS to the liquid scintillation fluid Opti-fluor (Packard, Groningen, The Netherlands) and counted in a liquid scintillation counter (Betamatic, Kontron). Cell-associated Dx was expressed in pmol/10<sup>6</sup> cells. Cellular Dx contents were determined after correction for immediate binding (0 min, 0°C). Total cell-associated Dx was 15% of the total amount of added Dx at maximum. For efflux experiments sensitive and resistant cells were loaded with 2 and 16  $\mu$ M Dx, respectively, for 90 min at 37°C. After loading, the cells were washed three times with ice-cold PBS, resuspended in medium to a concentration of 0.3  $\times$  10<sup>6</sup> cells/ml, and thereafter incubated at 37°C in the



**Fig. 1.** Potentiating effects of Bp and Vp on Dx-induced cytotoxicity in a Dx-sensitive (A2780) and -resistant (2780<sup>AD</sup>) human ovarian cancer cell line. Log-phase cells were exposed continuously at 37°C for 48 h (A2780) or 72 h (2780<sup>AD</sup>) at different concentrations of Dx in the presence or absence of Bp and Vp at the indicated concentrations: ●—●, A2780 control, without Bp or Vp; ■—■, A2780 plus 6  $\mu$ M Bp; ▲—▲, A2780 plus 10  $\mu$ M Vp; ○—○, 2780<sup>AD</sup> control, without Bp or Vp; □—□, 2780<sup>AD</sup> plus 4  $\mu$ M Bp; △—△, 2780<sup>AD</sup> plus 6  $\mu$ M Bp; ▽—▽, 2780<sup>AD</sup> plus 2  $\mu$ M Vp

**Table 1.** Dose-modifying factor (DMF) of different concentrations of Bp and Vp on Dx cytotoxicity to the human ovarian cancer cell line 2780<sup>AD</sup> following continuous or intermittent exposure

Blocker	Concentration ( $\mu$ M)	DMF	
		Continuous exposure	Intermittent exposure <sup>a</sup>
Bp	1	1.0 ( $\pm$ 0.1) <sup>b</sup>	1.0 ( $\pm$ 0.1)
Vp	1	2.1 ( $\pm$ 0.3)	2.7 ( $\pm$ 0.3)
Bp	2	2.5 ( $\pm$ 1.2)	2.8 ( $\pm$ 1.4)
Vp	2	3.6 ( $\pm$ 0.7)	3.7 ( $\pm$ 1.4)
Bp	4	8.7 ( $\pm$ 3.5)	8.7 ( $\pm$ 1.5)
Vp	4	7.2 ( $\pm$ 1.5)	8.4 ( $\pm$ 2.2)

<sup>a</sup> 2 h exposure to Dx; 4 h exposure to Bp or Vp

<sup>b</sup> Mean ( $\pm$  SD). Values taken from at least three independent experiments

presence or absence of blockers. At different times samples were diluted with 5 ml of ice-cold PBS, centrifuged, and counted as described above.

## Results

### Effects of Bp and Vp on Dx-induced growth inhibition in 2780<sup>AD</sup> and A2780 cells

When human ovarian cancer cells were exposed continuously to Dx, IC<sub>50</sub> values of 3.1 ( $\pm$ 0.6)  $\mu$ M (mean  $\pm$  SD;  $n$  = 6) and 0.022 ( $\pm$ 0.008)  $\mu$ M ( $n$  = 3) were observed, respectively, for 2780<sup>AD</sup> and A2780 cells (resistance factor 141), which is in rather good agreement with those previously reported by Louie et al. [5]. Figure 1 shows the effects of Bp on Dx-induced growth inhibition of these cells. In 2780<sup>AD</sup> cells, the blocker potentiated Dx cytotoxicity at different Dx concentrations in a dose-dependent way. Results obtained at low concentrations of Bp (1–4  $\mu$ M) are summarized in Table 1. Bp concentrations of up to 6  $\mu$ M failed to enhance Dx effects to a significant extent in the sensitive A2780 cells (Fig. 1). The effects of Bp on Dx-induced cytotoxicity were compared with those of Vp, and a dose-effect relationship for the latter could also be demon-

relationship for the latter could also be demonstrated. Table 1 shows that Bp is as effective as Vp at  $4\ \mu\text{M}$ , but the latter is more effective at concentrations of 1 and  $2\ \mu\text{M}$ . Statistical analysis (Student's *t*-test) showed that  $4\ \mu\text{M}$  Bp had a significantly greater effect on Dx cytotoxicity than  $1\ \mu\text{M}$  Vp ( $P < 0.01$ ) or  $2\ \mu\text{M}$  Vp ( $P < 0.01$ ). As is the case of Bp, Vp had only minor effects on Dx cytotoxicity in A2780 cells (an example at  $10\ \mu\text{M}$  Vp is shown in Fig. 1). Continuous exposure to Bp alone was found to be cytotoxic, especially in resistant cells. For example, Bp at  $4\ \mu\text{M}$  caused 27% growth inhibition in 2780<sup>AD</sup> cells and only 10% in A2780 cells, whereas Vp alone had a lower effect, with 16% growth inhibition in 2780<sup>AD</sup> cells at  $10\ \mu\text{M}$ . With the alternative protocol, which limited drug exposure time to eliminate any potential interference by the blockers themselves, an  $\text{IC}_{50}$  value of  $22 (\pm 18)\ \mu\text{M}$  (mean  $\pm$  SD;  $n = 5$ ) was observed for 2780<sup>AD</sup> cells, with no cytotoxicity for Bp (or Vp) alone, up to a concentration of  $8.5\ \mu\text{M}$ . From Table 1 it may be concluded that the order of potency of the blockers with intermittent exposure is similar to that observed with continuous incubation. Deletion of the additional incubation period with Bp, after the combined Dx-Bp incubation, resulted in a decrease of DMF with a factor of about 1.5, which probably reflects the effects of Bp on Dx efflux.

#### Effects of Bp and Vp on Dx accumulation and efflux in 2780<sup>AD</sup> and A2780 cells

The effects of Bp on Dx accumulation and efflux were studied in order to investigate whether there is a correlation between Dx cytotoxicity and drug accumulation. In Fig. 2, the time-course of accumulation in A2780 and 2780<sup>AD</sup> cells and the effects of  $8.5\ \mu\text{M}$  Bp on this process are shown. Accumulation of Dx in 2780<sup>AD</sup> cells was one-third of that in A2780 cells:  $52.4 (\pm 13.7) \times 10^6$  pmol/ $10^6$  cells (mean  $\pm$  SD;  $n = 7$ ) for the former versus  $153.7$

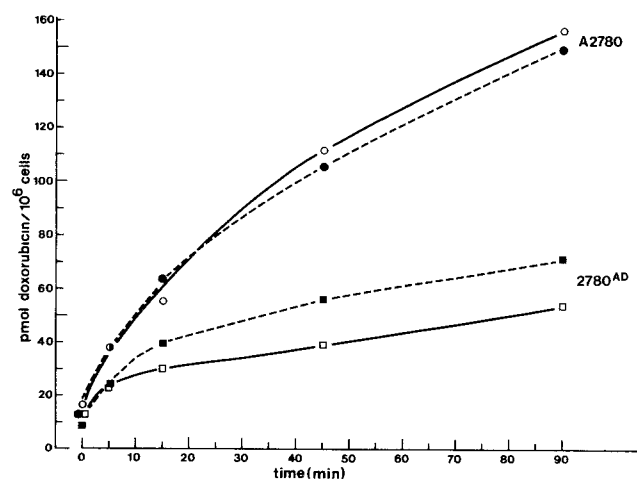


Fig. 2. A representative experiment investigating the time-course of Dx accumulation in sensitive (A2780) and resistant (2780<sup>AD</sup>) human ovarian carcinoma cells and the effect of Bp. Cells ( $10^6/\text{ml}$ ) were incubated at  $37^\circ\text{C}$  with  $2\ \mu\text{M}$  Dx in the presence or absence of  $8.5\ \mu\text{M}$  Bp. At the indicated times, duplicate samples were taken and processed.  $\circ$ — $\circ$ , A2780 control;  $\bullet$ — $\bullet$ , A2780 plus  $8.5\ \mu\text{M}$  Bp;  $\square$ — $\square$ , 2780<sup>AD</sup> control;  $\blacksquare$ — $\blacksquare$ , 2780<sup>AD</sup> plus  $8.5\ \mu\text{M}$  Bp

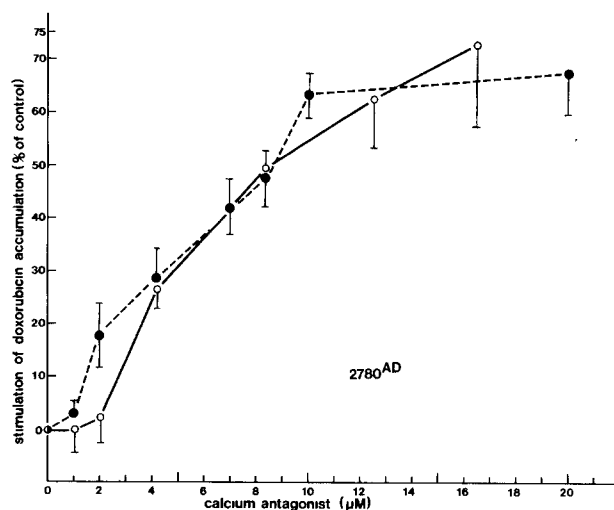


Fig. 3. Stimulation of Dx accumulation in 2780<sup>AD</sup> cells by Bp and Vp. Cellular accumulation was determined after 90 min at  $37^\circ\text{C}$  in the presence or absence of different concentrations of Bp and Vp. Symbols represent the means ( $\pm$  SE) of 5 experiments, except for 1 and  $2\ \mu\text{M}$  Bp and Vp (3 experiments).  $\circ$ — $\circ$ , Bp;  $\bullet$ — $\bullet$ , Vp

( $\pm 16.7$ )  $\times 10^6$  pmol/ $10^6$  cells ( $n = 4$ ) for the latter. Figure 3 shows that in 2780<sup>AD</sup> cells, Bp and Vp cause a dose-dependent increase in cellular Dx after 90 min at  $37^\circ\text{C}$ . Whereas the two compounds enhanced accumulation to the same extent in the concentration range of 4– $16.5\ \mu\text{M}$ , Vp appeared more potent than Bp at concentrations below  $4\ \mu\text{M}$ . Statistical analysis (Student's *t*-test) revealed that the

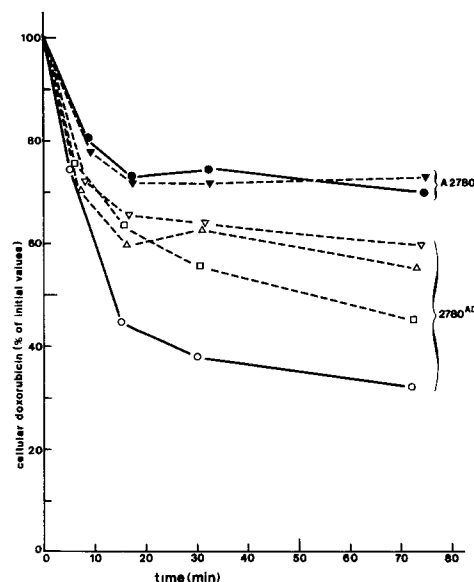


Fig. 4. Efflux of Dx from preloaded A2780 and 2780<sup>AD</sup> cells and the effects of Bp. Cells were preloaded with Dx for 90 min at  $37^\circ\text{C}$ , and after extensive washing were suspended in drug-free medium and incubated further at  $37^\circ\text{C}$  in the presence or absence of different concentrations of Bp. Samples were withdrawn at specific times. Initial amounts of cellular Dx were  $181\ \text{pmol}/10^6$  cells for A2780 and  $92\ \text{pmol}/10^6$  cells for 2780<sup>AD</sup> (loaded with 2 and  $16\ \mu\text{M}$  Dx, respectively).  $\bullet$ — $\bullet$ , A2780 control;  $\blacktriangledown$ — $\blacktriangledown$ , A2780 plus  $12.5\ \mu\text{M}$  Bp;  $\circ$ — $\circ$ , 2780<sup>AD</sup> control;  $\square$ — $\square$ , 2780<sup>AD</sup> plus  $4\ \mu\text{M}$  Bp;  $\triangle$ — $\triangle$ , 2780<sup>AD</sup> plus  $8.5\ \mu\text{M}$  Bp;  $\nabla$ — $\nabla$ , 2780<sup>AD</sup> plus  $12.5\ \mu\text{M}$  Bp

**Table 2.** Doxorubicin cytotoxicity and accumulation in AUXB1 and CH<sup>R</sup>C5 cells, and the effects of Bp and Vp<sup>a</sup>

Modulating compound	Concentration (μM)	DMF		Drug content (pmol/10 <sup>6</sup> cells)	
		AUXB1	CH <sup>R</sup> C5	AUXB1	CH <sup>R</sup> C5
None	—	1	1	169.3 (100)	50.4 (100)
Bp	1	N.D.	0.9	N.D.	50.2 (100)
Vp	1	N.D.	3.4	N.D.	63.0 (125)
Bp	2	1.4	8.7	218.4 (129)	N.D.
Vp	2	1.8	24.1	255.3 (151)	70.2 (139)
Bp	4	1.4	74.0	224.7 (133)	72.9 (145)
Vp	4	2.0	104.0	260.7 (154)	90.5 (180)
Bp	8.5	N.D.	N.D.	N.D.	99.0 (196)
Vp	8.5	N.D.	N.D.	N.D.	111.0 (220)
Bp	16.5	N.D.	260	241.9 (143)	137.8 (273)
Vp	16.5	N.D.	236	249.5 (147)	145.4 (289)
Bp	32	N.D.	325	N.D.	N.D.
Vp	32	N.D.	300	N.D.	N.D.

Percentages of control shown in parentheses

N.D., not determined

<sup>a</sup> Results are means of two dose-response experiments with cells from different cell passages

effect of 4 μM Bp was significantly greater than that of 1 μM Vp ( $P < 0.01$ ). In A2780 cells, even a concentration of 12.5 μM Bp failed to enhance Dx accumulation: 141.0 ( $\pm 23.5$ )  $\times 10^6$  pmol/10<sup>6</sup> cells (mean  $\pm$  SD;  $n = 4$ ) as against 153.7 ( $\pm 16.7$ )  $\times 10^6$  pmol/10<sup>6</sup> cells ( $n = 4$ ) in control cells. Overall, the differences in the pharmacological effects of the two compounds are in good agreement with the biological effects. Since it has repeatedly been shown that calcium antagonists and calmodulin inhibitors stimulate drug accumulation in resistant cells by inhibiting drug efflux, we also studied the effects of Bp on drug efflux. In Fig. 4 a representative experiment is shown, in which Dx efflux from preloaded A2780 and 2780<sup>AD</sup> cells was studied. Efflux from resistant cells occurs faster than that from sensitive cells. Whereas A2780 cells still retained 60%–80% after 45 min of incubation, 2780<sup>AD</sup> cells retained only 30%–40% (Fig. 4). Bp inhibited the efflux from 2780<sup>AD</sup> cells in a concentration-dependent manner, but did not affect the efflux from A2780 cells. The presence of Bp during the Dx-efflux phase in 2780<sup>AD</sup> cells after a short-term (2 h) exposure to Dx may significantly affect DMF values, as discussed in the preceding section.

#### *Effects of Bp and Vp on Dx-induced growth inhibition and Dx accumulation in CH<sup>R</sup>C5 and AUXB1 cells*

The effects of Bp and Vp were also studied in CHO cell lines. In the following experiments under continuous drug exposure, the colchicine-resistant CH<sup>R</sup>C5 cell line exhibited a sensitivity to Dx which was 195 times less than that of AUXB1, with an IC<sub>50</sub> of 11.1 ( $\pm 3.3$ ) μM (mean  $\pm$  SD;  $n = 6$ ) for CH<sup>R</sup>C5 cells and an IC<sub>50</sub> of 0.057 ( $\pm 0.038$ ) μM ( $n = 7$ ) for AUXB1 cells. Both compounds preferentially exhibited growth inhibition in the resistant cells (90% and 80% inhibition with 4 μM Bp and Vp, respectively, but only 30% and 5%, respectively, in AUXB1 cells). Both Bp and Vp considerably potentiated Dx cytotoxicity in AUXB1 cells, with a DMF of about 5 for 4 μM of each of the blockers. In order to circumvent the cytotoxic effects of the two compounds in CH<sup>R</sup>C5 cells, the alternative short-term incubation procedure was applied for this cell line. These experiments showed no substantial growth in-

hibition of CH<sup>R</sup>C5 cells following exposure up to 16.5 μM of the calcium antagonist. The effects of Bp and Vp on Dx cytotoxicity to CH<sup>R</sup>C5 (IC<sub>50</sub>: 120 μM) and AUXB1 (IC<sub>50</sub>: 0.5 μM) cells in these short-term exposure experiments are illustrated in Table 2. At each specific, low concentration (1, 2, and 4 μM) used for the CH<sup>R</sup>C5 cells, Vp proved to have a greater potency than Bp. However, the effect of each concentration of Bp used was consistently greater than that of the next lower concentration of Vp. Moreover, Dx resistance in CH<sup>R</sup>C5 cells could be completely overcome by high concentrations (16.5 μM) of the two compounds. From Table 2 it is clear that DMF values in AUXB1 cells were considerably lower than those observed under continuous incubation of the drugs.

Both Bp and Vp strongly enhanced Dx accumulation in CH<sup>R</sup>C5 cells (Table 2). At the highest concentration of 16.5 μM, both blockers were able to enhance accumulation of Dx to levels close to those in AUXB1 (Table 2). Vp appears to be more potent than Bp at all concentrations used. Generally, the order of potency of the different concentrations of both blockers parallels that obtained in the cytotoxicity experiments. Whereas Dx accumulation was enhanced in AUXB1 by Bp, the effect of Vp was slightly greater (Table 2). Almost maximal enhancement was reached with each compound at a concentration as low as 2 μM (Table 2).

#### **Discussion**

Calcium channel-blocking agents and calmodulin inhibitors have been studied extensively to determine their reversing effect on anticancer drug resistance [4]. Vp has been studied most extensively in this respect [9]. Although animal experiments with Vp have been promising [9], clinical trials have not been successful: whereas plasma concentrations of 1 μM or less have been achieved [3], such low concentrations have rarely been used in *in vitro* studies [9]. The present study involving the calcium channel blocker Bp was undertaken because it has been shown that plasma concentrations of this compound as high as 2–4 μM can be achieved clinically [2]. Such concentrations

of Bp were shown to cause a partial reversal of Dx resistance in 2780<sup>AD</sup> and CH<sup>R</sup>C5 cells.

In addition, a moderate-to-high growth inhibition was observed for the calcium channel blockers, in particular Bp, in the resistant cell lines at clinically achievable concentrations. The growth inhibition was most pronounced in CH<sup>R</sup>C5 cells and moderate in 2780<sup>AD</sup> cells, whereas drug-sensitive cells were affected to a lesser extent. The cause of this phenomenon is unknown, but one may speculate that this differential sensitivity of drug-sensitive and -resistant cells to the blockers may have clinical relevance in the treatment of drug-resistant cells. Since our primary goal was to compare the ability of Bp and Vp to restore drug sensitivity in resistant cells, we also implemented a short-term incubation, in which the inherent effects of Bp and Vp on cell growth were largely eliminated. Moreover, short-term incubations with Dx probably reflect the *in vivo* situation following bolus injections better than continuous incubations with this drug. Whereas with both incubation schedules Vp and Bp were equipotent in 2780<sup>AD</sup> cells at concentrations of 4  $\mu$ M and higher, Vp was more active in short-term incubation experiments with CH<sup>R</sup>C5 cells (these cells have not been subjected to continuous exposure at these concentrations). The effects of both blockers were compared at clinically achievable plasma concentrations of 2–4  $\mu$ M for Bp and 0.5–1  $\mu$ M for Vp. This comparison indicates that 4  $\mu$ M Bp is 3–4 times more active than 1  $\mu$ M Vp in 2780<sup>AD</sup> cells (Table 1), while this factor is over 20 for CH<sup>R</sup>C5 cells (Table 2). Even at a concentration of 2  $\mu$ M, Vp was less effective than 4  $\mu$ M Bp (a factor of 2.4 and 3.1 for 2780<sup>AD</sup> and CH<sup>R</sup>C5, respectively). The DMFs of 2.1–3.7 at 1–2  $\mu$ M observed for Vp in 2780<sup>AD</sup> cells were less than those of 3–6 at 0.5–1  $\mu$ M previously reported for this cell line [7]. This discrepancy can probably be attributed to the different cytotoxicity assays, growth inhibition vs inhibition of colony formation, used in the two studies.

Although the variable potency of Bp compared to that of Vp is not understood (Bp is less potent than Vp at concentrations below 4  $\mu$ M and equipotent to Vp at concentrations of 4  $\mu$ M or higher in 2780<sup>AD</sup> cells), the results at least indicate that the common procedure in experiments on drug resistance – comparing the modifying activities of drugs at an arbitrary concentration – should be subject to criticism.

Despite the fact that previous experiments by Louie et al. were performed at a 13-fold lower concentration of Dx [5], the differences we found in Dx accumulation between drug-sensitive and -resistant human cell lines (showing a threefold greater accumulation of Dx in A2780 than in 2780<sup>AD</sup> cells) are similar. Because it is difficult to comprehend that such a small difference in accumulation could result in such a large difference in sensitivity, it has been argued that other mechanisms might be involved in drug resistance [6]. It is interesting that resistance could be completely overcome in CH<sup>R</sup>C5 cells and that this correlated well with the increase in accumulation up to the AUXB1 level. In the former cell line, an altered transport mechanism therefore seems to account fully for resistance. In the human cell line 2780<sup>AD</sup>, complete reversal of resistance and of the accumulation defect have not been possible; therefore, an alteration of transport may not be the sole mechanism of resistance in this cell line. In another set of experiments (not presented in this paper), Bp (8.5  $\mu$ M) sig-

nificantly, though slightly, enhanced Dx accumulation in 2780<sup>AD</sup> cells exposed to only 0.2  $\mu$ M Dx, from a baseline level of 9 pmol/10<sup>6</sup> cells to about 12 pmol/10<sup>6</sup> cells. Surprisingly, despite this limited increase, continuous exposure to a combination of these drugs resulted in 50% growth inhibition, similar to that obtained with 2  $\mu$ M Dx alone. However, in the latter control experiment, 2  $\mu$ M Dx resulted in an accumulation as high as 52 pmol/10<sup>6</sup> cells. These results support the hypothesis that Bp may enhance Dx cytotoxicity not only by stimulating its accumulation by the cells, but also by some other mechanism, perhaps through redistribution of Dx inside the cells – enabling it to reach targets more conducive to cytotoxicity. This theory is supported by the ability of Bp to increase nuclear Dx fluorescence relative to cytoplasmic Dx fluorescence in 2780<sup>AD</sup> and CH<sup>R</sup>C5 cells (details of the relationship between drug cytotoxicity, accumulation, and intracellular distribution will be presented in another paper).

In conclusion, our results with the calcium channel-blocking agent Bp indicate that this compound can partly reverse Dx resistance and that it is more effective than Vp at clinically achievable concentrations. Bp is thus a promising agent for *in vivo* studies on the reversal of drug resistance.

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