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FACTORS GOVERNING THE MODULATION OF VINCA-ALKALOID RESISTANCE IN DOXORUBICIN-RESISTANT CELLS BY THE CALMODULIN INHIBITOR TRIFLUOPERAZINE*

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Abstract—Calmodulin inhibitors enhance the cytotoxic effects of doxorubicin (DOX) in DOX-resistant (P388/DOX) P388 mouse leukemia cells by augmenting cellular accumulation and retention of drug. In P388/DOX cells which are cross-resistant to vinblastine (VLB) and vincristine (VCR), cell kill following treatment with VLB and VCR alone was evident only after 12 hr of treatment. Additionally, the 2- to 10-fold increase in cytotoxicity of the vinca alkaloids in the presence of 2 and 4 μM trifluoperazine (TFP) was observed only in P388/DOX cells treated for 12 hr, but not for 3 or 6 hr. However, in DOX-sensitive (P388/S) P388 mouse leukemia cells, cytotoxic effects of VCR but not VLB were apparent after treatment for 3 hr, and cell kill with VLB and VCR was enhanced 2- to 20-fold in the presence of 2 and 4 μM TFP following treatment for 12 hr. Cellular accumulation of [^{3}H]VLB in P388/DOX cells was 12-fold lower than in similarly treated P388/S cells and, in the presence of 2 and 4 μM TFP, cellular VLB levels were enhanced 1.3- to 2.0-fold in P388/S cells and 2- to 8-fold in P388/DOX cells. The effect of TFP in increasing cellular retention of [^{3}H]VLB was more apparent with P388/DOX cells, and retention of [^{3}H]VLB in the presence of 4 μM TFP was enhanced <1.5-fold and >4-fold in P388/S and P388/DOX cells respectively. Results from this study and our earlier observations with DOX and TFP in P388/DOX cells demonstrate that: (1) TFP potentiates the cytotoxicity of VLB and VCR in P388/S and P388/DOX cells by augmenting drug accumulation and retention; (2) enhanced cell kill in the presence of TFP with P388/DOX cells is apparent at 1 hr for DOX vs 12 hr for VLB and VCR; and (3) in P388/S cells, TFP has a more striking effect on the cellular accumulation, retention and cytotoxicity of VLB and VCR rather than DOX.

The vinca alkaloids vinblastine (VLB‡) and vincristine (VCR) obtained from the periwinkle plant, *Catharanthus roseus* G. Don, are widely used in the chemotherapy of human malignancies [1]. Although VCR differs from VLB only in the substitution of a formyl group for the N-methyl group in the vindoline moiety, the two agents differ significantly in their cellular pharmacokinetics, spectrum of antitumor activity, and toxicity [1–5]. The clinical management of tumors with acquired drug resistance continues to be a serious challenge, in spite of the major advances in the chemotherapy of malignancies [6]. In tumor model systems with acquired-resistance to doxorubicin (DOX) and daunorubicin (DAU) or VCR and VLB, a significant degree of cross-resistance between the anthracyclines and vinca alkaloids is observed, in spite of differences in the mechanism of action between these two classes of cytotoxic drugs [1, 2, 7–11]. Cellular pharmacokinetic studies have indicated that tumor cell resistance to DAU, DOX, VCR and VLB is due primarily to reduced drug accumulation and/or impaired drug retention [8, 11–14]. Reduced drug accumulation in the resistant sublines has been suggested to be due to alterations in membrane permeability, possibly related to the presence of a M_r 170,000 plasma membrane glycoprotein [15]. The concept of a permeability barrier has been also supported by experimental evidence demonstrating that a perturbation of membrane permeability by detergents, like Tween 80, can significantly enhance drug sensitivity in the resistant cells [16, 17].

Acquired resistance to DOX and VCR in mouse leukemia P388 cells can be significantly overcome by calmodulin inhibitors [18–20] and calcium antagonists [20–22] which augment cellular accumulation and/or retention of DOX and VCR. More recently [23] we have demonstrated that, in DOX-resistant P388 mouse leukemia cells, trifluoperazine has a differential effect on the cellular levels and cytotoxicities of anthracyclines, which differ from DOX in their cellular pharmacokinetics, and the objective of this study was to determine the characteristics of cross-resistance to VLB and VCR in DOX-resistant cells and the role of calmodulin inhibitors in modulating resistance. Specifically, using the DOX-sensitive (P388/S) and DOX-resistant (P388/DOX) P388 mouse leukemia model system, the effects of the calmodulin inhibitor trifluoperazine on the cellular accumulation and retention of VLB and cytotoxicities of VLB and VCR were determined.

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‡ Abbreviations: VLB, vinblastine; VCR, vincristine; DOX, doxorubicin; DAU, daunorubicin; TFP, trifluoperazine dihydrochloride; and FBS, fetal bovine serum.

MATERIALS AND METHODS

The source and conditions for maintenance *in vitro* of P388/S and P388/DOX cells were similar to those reported previously [18, 24]. Trifluoperazine dihydrochloride (TFP) was a gift from Dr. Carl Kaiser, Smith Kline & French Laboratories, Philadelphia, PA. [^{3}H-(G)]Vinblastine sulfate (sp. act. 5 Ci/mmole), purity > 95% based on high performance liquid chromatographic analysis, was obtained from Moravek Biochemicals Inc., Brea, CA.

Vinca-alkaloid cytotoxicity in vitro. All cytotoxicity studies were carried out using a soft-agar colony forming assay. Stock solutions of VLB, VCR and TFP were made in sterile glass distilled water and working dilutions prepared in RPMI 1640 supplemented with 25 mM *N*-2-hydroxyethylpiperazine-*N*-ethanesulfonic acid buffer (M.A. Bioproducts, Walkersville, MD) and 10% fetal bovine serum (FBS) (Sterile Systems, Inc., Logan, UT) for VLB and VCR, and serum-free RPMI 1640 for TFP. P388/S and P388/DOX cells in RPMI 1640 supplemented with 10% FBS were treated with various concentrations of VLB and VCR in the absence and presence of TFP. The concentrations of VLB and VCR utilized for the various lengths of treatment were as follows: VLB and VCR, 5.5 to 110 nM for P388/S cells and 11 to 2200 nM for P388/DOX cells in 3-hr drug exposure experiments; and VLB and VCR, 1.1 to 5.5 nM for P388/S cells and 11 to 110 nM for P388/DOX cells in 12-hr drug exposure experiments. The concentration of TFP for the 3-hr treatment was 4 μM, and for 12-hr drug exposure 2 and 4 μM TFP were used. Following the desired length of treatment, the cells were centrifuged (80 *g*), washed twice with drug-free RPMI 1640 supplemented with 10% FBS, and plated in triplicate at a density of 1.5×10^4 cells/petri dish in 35 × 10 mm petri dishes. The plating medium used was RPMI 1640 supplemented with 20% FBS, 10 μM 2-mercaptoethanol, conditioned medium from culture supernatant fractions of P388/S or P388/DOX cells, and 0.3% agar. Following incubation for 120 hr in a humidified 5% CO_2 plus 95% air atmosphere, colonies (>50 cells) in untreated control and treated plates were counted in an Omnicon Feature Analysis System II (Bausch & Lomb, Rochester, NY). The plating efficiency of control untreated P388/S and P388/DOX cells under these conditions was approximately 30%. In cytotoxicity studies involving 12 hr of drug treatment, cell counts were determined by trypan blue dye-exclusion at the end of drug exposure, and aliquots of cells from control and treated cultures were washed and plated (1.5×10^4 trypan blue dye-excluding cells/petri dish) in agar as outlined earlier.

Cellular accumulation of vinblastine in vitro. P388/S and P388/DOX cells in RPMI 1640 supplemented with 10% FBS were treated with 11, 22 and 44 nM VLB ([^{3}H-(G)]VLB and unlabeled VLB) in the absence and presence of 2 and 4 μM TFP at 37°. Duplicate 1-ml aliquots of cells (2×10^6) retrieved at the end of 1 hr of incubation were centrifuged (530 *g*) and washed twice with 7 ml of ice-cold 0.85% sodium chloride solution. The cell pellet after the final wash was drained, reconstituted in 100 μl of glass distilled water and digested with 0.5 ml of Protosol (New England Nuclear, Inc., Boston, MA) for 16 hr at room temperature, followed by 2 hr at 54° in a shaker-water bath (Forma Scientific, Marietta, OH). The digest was then mixed with 10 ml of Econofluor (New England Nuclear) and counted in a Beckman LS 7500 liquid scintillation counter (Beckman Instruments, Fullerton, CA). Counting efficiency for tritium in the Protosol-containing samples was 52%, and cellular levels of VLB were expressed as picomoles [^{3}H]VLB/2×10^6 cells.

Cellular retention of vinblastine in vitro. P388/S and P388/DOX cells in RPMI 1640 supplemented with 10% FBS were pretreated for 1 hr at 37° with VLB ([^{3}H-(G)]VLB and unlabeled VLB) at concentrations of 5.5 nM VLB for P388/S cells and 22 to 44 nM VLB for P388/DOX cells, in the absence and presence of 4 μM TFP. Cells were then centrifuged, resuspended in VLB-free medium (RPMI 1640 supplemented with 10% FBS) in the absence and presence of 4 μM TFP, and incubated at 37°. Duplicate aliquots of cells (2×10^6 cells/sample) retrieved at the end of the 1-hr pretreatment drug accumulation phase, and at the end of 15, 30, 60, 90 and 120 min during the retention phase, were centrifuged (530 *g*) and washed twice with 7 ml of ice-cold 0.85% sodium chloride solution. Samples were then processed for determining cellular levels of [^{3}H-(G)]VLB as outlined earlier under accumulation experiments.

RESULTS

Effect of TFP on the cytotoxicity of vinca alkaloids in vitro. The effect of TFP on the cytotoxicity of VLB and VCR in P388/S cells treated for 3 hr is shown in Table 1. It is apparent from the data that treatment of P388/S cells with VLB at concentrations of 5.5 to 110 nM for 3 hr was ineffective, and in the presence of TFP no enhancement in cytotoxic effects was observed. In contrast, the survival of P388/S cells treated with VCR alone for 3 hr was dose-dependent over the concentration range studied, and a 1.6- to 2-fold enhancement in cytotoxicity in the

Table 1. Effect of trifluoperazine (TFP) on the cytotoxicity *in vitro* of vinblastine and vincristine in doxorubicin-sensitive (P388/S) P388 mouse leukemia cells treated for 3 hr

Drug concentration	Survival* (% of control) Vinblastine†	Vincristine†
4 μM TFP	100 ± 0	100 ± 0
5.5 nM	89 ± 5	89 ± 1
5.5 nM + 4 μM TFP	95 ± 5	54 ± 4
11 nM	88 ± 6	70 ± 3
11 nM + 4 μM TFP	92 ± 5	35 ± 4
55 nM	90 ± 6	25 ± 3
55 nM + 4 μM TFP	92 ± 6	21 ± 1
110 nM	85 ± 4	18 ± 1
110 nM + 4 μM TFP	90 ± 6	16 ± 1

* Values are expressed as mean ± S.E. of triplicate experiments. Survival is based on colony counts.

† P388/S cells were treated with various concentrations of vinblastine and vincristine in the absence and presence of 4 μM trifluoperazine for 3 hr, washed, and plated in soft-agar.

Table 2. Effect of trifluoperazine (TFP) on the cytotoxicity *in vitro* of vinblastine and vincristine in doxorubicin-sensitive (P388/S) P388 mouse leukemia cells treated for 12 hr

Drug concentration	Survival* (% of control) Vinblastine†	Vincristine†
2 μM TFP	99 ± 1	99 ± 1
4 μM TFP	94 ± 1	94 ± 1
1.1 nM	90 ± 3	84 ± 11
1.1 nM + 2 μM TFP	57 ± 14	8 ± 1
1.1 nM + 4 μM TFP	38 ± 13	2 ± 1
2.75 nM	36 ± 5	21 ± 3
2.75 nM + 2 μM TFP	2.8 ± 1.1	1.1 ± 0.1
2.75 nM + 4 μM TFP	3.2 ± 0.9	1.1 ± 0.3
5.5 nM	2.3 ± 0.9	1.6 ± 0.4
5.5 nM + 2 μM TFP	2.6 ± 0.5	0.9 ± 0.4
5.5 nM + 4 μM TFP	3.3 ± 0.4	0.8 ± 0.4

* Values are expressed as mean ± S.E. of triplicate experiments. Survival is based on colony counts.

† P388/S cells were treated with various concentrations of vinblastine and vincristine in the absence and presence of 2 and 4 μM trifluoperazine for 12 hr, washed, and plated in soft-agar.

presence of 4 μM TFP was observed at VCR doses of 5.5 and 11 nM. Higher concentrations of 55 and 110 nM VCR alone induced an 80% kill in P388/S cells, and any further enhancement in cytotoxic effects due to TFP was not observed at these VCR doses. Similar to data with P388/S cells, VLB at concentrations as high as 2200 nM in the absence and presence of 4 μM TFP were not cytotoxic to P388/DOX cells treated for 3 hr (data not presented). However, unlike the cell kill observed in P388/S cells treated with VCR and VCR plus 4 μM TFP for 3 hr, in P388/DOX cells VCR at concentrations of 11 to 2200 nM was noncytotoxic in the absence or presence of 4 μM TFP (unpublished results).

Table 3. Effect of trifluoperazine (TFP) on the cytotoxicity *in vitro* of vinblastine and vincristine in doxorubicin-resistant (P388/DOX) P388 mouse leukemia cells treated for 12 hr

Drug concentration	Survival (% of control) Vinblastine*	Vincristine*
2 μM TFP	98 ± 0†	99 ± 1‡
4 μM TFP	99 ± 1	99 ± 1
11 nM	98 ± 1	99 ± 1
11 nM + 2 μM TFP	52 ± 7	98 ± 2
11 nM + 4 μM TFP	9 ± 2	66 ± 9
27.5 nM	95 ± 0‡	98 ± 1
27.5 nM + 2 μM TFP	6.5 ± 0.5‡	82 ± 4
27.5 nM + 4 μM TFP	8 ± 1.5‡	9 ± 2
55 nM	51 ± 3‡	98 ± 1
55 nM + 2 μM TFP	6 ± 1‡	18 ± 6
55 nM + 4 μM TFP	12.5 ± 0.5‡	8 ± 2
110 nM	11.5 ± 0.5‡	92 ± 1
110 nM + 2 μM TFP	8.5 ± 0.5‡	6 ± 1
110 nM + 4 μM TFP	19 ± 0‡	8 ± 3
550 nM		4 ± 1

* P388/DOX cells were treated with various concentrations of vinblastine and vincristine in the absence and presence of 2 and 4 μM trifluoperazine for 12 hr, washed, and plated in soft-agar.

† Values are expressed as mean ± S.E. of triplicate experiments. Survival is based on colony counts.

‡ Values are from triplicate plates in duplicate experiments.

Results demonstrating the effects of 2 and 4 μM TFP on the cytotoxicity of vinblastine and vincristine in P388/S cells treated for 12 hr are shown in Table 2. The data suggest that, with 12 hr of continuous treatment, VCR and VLB are equitoxic and cell kill was dependent on drug concentration. Additionally, the enhancement (2- to 20-fold) in cytotoxic effects due to TFP was observed only at the lower concentrations of VLB and VCR (1.1 and 2.75 nM) and was independent of the concentration of TFP.

The cytotoxic effects of VLB and VCR in the absence and presence of 2 and 4 μM TFP in P388/DOX cells treated for 12 hr are presented in Table 3. In P388/DOX cells treated with VLB alone, cytotoxic effects were concentration dependent and the 2- to 10-fold enhancement in cell kill was fairly independent of TFP concentration (2 μM ≅ 4 μM). Cell kill in P388/DOX cells treated with 11 to 110 nM of VCR alone was minimal, and significant cytotoxicity (<5% survival) was observed only at a concentration of 550 nM VCR. Similar to the results with VLB, a 2- to 10-fold enhancement in cytotoxicity of VCR in the presence of TFP was observed. Additionally, the effects of TFP were found to be concentration dependent (4 μM > 2 μM) only at VCR concentrations of 11 to 55 nM.

Effect of TFP on cellular vinblastine accumulation in vitro. In Fig. 1 are shown the effects of 2 and 4 μM TFP on the accumulation of VLB in P388/S and P388/DOX cells treated for 1 hr with 11, 22 and 44 nM VLB. Cellular accumulation of VLB was >12-fold lower in P388/DOX cells than in similarly treated P388/S following treatment for 1 hr with 11, 22 and 44 nM VLB alone. In P388/S cells, a 1.3- to 2-fold enhancement in cellular accumulation of VLB was observed, following treatment with 11, 22 and 44 nM VLB in the presence of 2 and 4 μM TFP, and the magnitude of increase in cellular VLB levels due to TFP was lower at higher extracellular concentrations of VLB (44 nM < 11 nM). In P388/DOX cells, a more pronounced effect of TFP in enhancing cellular accumulation of VLB was observed. A 2-fold and an 8-fold increase in cellular VLB accumulation at TFP concentrations of 2 and 4 μM, respectively, were observed in P388/DOX cells treated with 11, 22 and 44 nM VLB. The cellular accumulation of VLB in P388/S and P388/DOX cells treated with 11 nM VLB in the absence and presence of 4 μM TFP for 3 and 6 hr was similar to data obtained at 1 hr.

Effect of TFP on cellular vinblastine retention in vitro. Since cellular drug retention is an important determinant of resistance, the effect of TFP on VLB retention in P388/S and P388/DOX cells was determined, and data from these studies for P388/DOX cells are shown in Fig. 2. The retention of VLB at the end of 90–120 min in P388/S and P388/DOX cells treated without TFP during the 1-hr accumulation phase and subsequent retention phase was ~20% of the drug initially accumulated. However, in P388/DOX cells treated for 1 hr with VLB plus 4 μM TFP during the accumulation phase, and subsequently incubated in RPMI 1640 supplemented with 10%

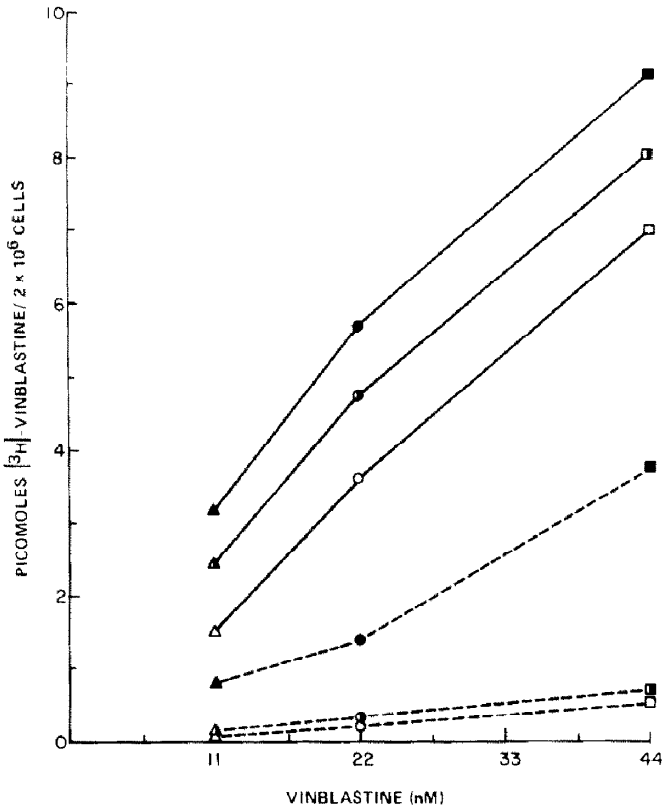

Fig. 1. Effect of TFP on the accumulation of [^{3}H]VLB in P388/S (——) and P388/DOX (- - -) cells. Each point is the mean value of triplicate experiments; the standard deviations were <15% of the means. Key: (△) 11 nM VLB alone; (▲) 11 nM VLB plus 2 μM TFP; (▲) 11 nM VLB plus 4 μM TFP; (○) 22 nM VLB; (◑) 22 nM VLB plus 2 μM TFP; (●) 22 nM VLB plus 4 μM TFP; (□) 44 nM VLB; (■) 44 nM VLB plus 2 μM TFP; and (■) 44 nM VLB plus 4 μM TFP.

FBS, the retention of VLB was >4-fold higher in the presence than in the absence of 4 μM TFP. In contrast, similar studies with P388/S cells, wherein the 1-hr accumulation of VLB was carried out with 4 μM TFP, and subsequent retention of VLB determined in RPMI 1640 supplemented with 10% FBS, in the absence and presence of 4 μM TFP, no remarkable difference in VLB retention due to TFP was observed (<1.5-fold increase).

DISCUSSION

Cellular resistance to DOX, DAU, VLB and VCR has been suggested to be due to reduced accumulation and/or retention of drug. Earlier studies attempting to circumvent cellular resistance to DOX and VCR have demonstrated that membrane perturbing agents, e.g. Tween 80, are capable of nonspecifically enhancing cellular drug accumulation and consequently cytotoxicity [16, 17]. More recently, calmodulin inhibitors and calcium antagonists have been demonstrated to enhance significantly the sensitivity to DOX and VCR in DOX-resistant and VCR-resistant tumor models respectively [18–20]. Although these studies have not demonstrated the involvement of a calcium-dependent and/or calmodulin-mediated process in the expression of resistance, experimental data indicate: (a) a relationship between potency of calmodulin inhibitors and enhancement of DOX sensitivity in DOX-resistant cells [19], and (b) the presence of elevated calcium levels in the resistant cells [25].

It is apparent from the data presented in this study that the P388/DOX cells are significantly cross-resistant to VLB and VCR. Cell kill following treatment for 12 hr with the vinca alkaloids in the absence of TFP (Table 3) indicates that P388/DOX cells are approximately 20- and 100-fold resistant to VLB and VCR respectively. However, when P388/DOX cells were treated for 12 hr with VLB and VCR in the presence of 4 μM TFP, no differential effects on cell kill or degree of resistance between the two vinca alkaloids were observed. These findings are particularly significant since VLB and VCR, although similar in their mechanisms of action, differ significantly in their cellular pharmacokinetics [3–5]. Recent *in vitro* and *in vivo* studies in a variety of model systems suggest the following differences between VLB and VCR in their cellular pharmacokinetics: (a) cellular accumulation and steady-state levels of VLB are more rapidly achieved than those of VCR; and (b) cellular retention of VCR is significantly higher than that of VLB [3–5]. Additionally, studies by Wilkoff and Dulmadge [26] and Jackson and Bender [27] have demonstrated that cytotoxic effects of VCR and VLB are significantly dependent on the concentration of drug and length of treatment, suggesting the requirement for minimum

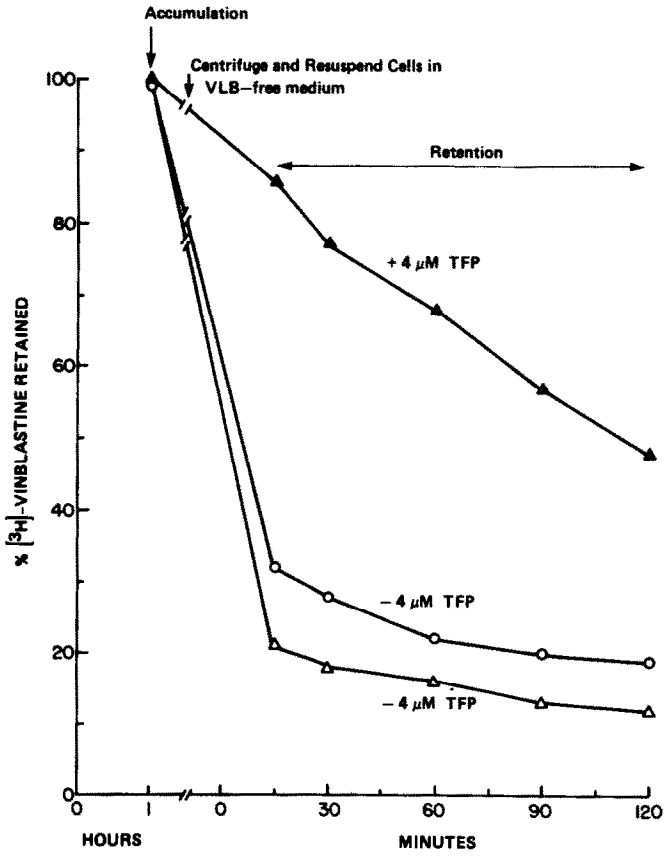

Fig. 2. Cellular retention of [^{3}H]VLB in P388/DOX cells in the absence and presence of TFP. Each point is the mean value of triplicate experiments; the standard deviations were <15% of the means. Key: (▲) [^{3}H]VLB accumulation and retention in the presence of 4 μM TFP; (△) [^{3}H]VLB accumulation in the presence of 4 μM TFP and retention in the absence of 4 μM TFP; and (○) [^{3}H]VLB accumulation and retention in the absence of 4 μM TFP.

threshold cellular drug levels for a cytotoxic effect. Based on this evidence and the cell cycle phase dependent specificity for cytotoxic effects of the vinca alkaloids, at least in P388/S cells, the cell kill with VCR but not VLB following 3-hr drug exposure is not surprising. In P388/S cells, increasing the length of drug exposure to 6 hr did favor significant cytotoxicity with VLB (unpublished results), and the concentrations of VCR needed to achieve equivalent cell kill were 100-fold lower than those of VLB. However, when drug treatment was increased to 12 hr, VLB and VCR were equitoxic to P388/S cells at similar concentrations (Table 2). Cytotoxic effects of VLB and VCR were enhanced 2- to 20-fold by TFP in P388/S cells, and when VLB alone was non-cytotoxic due to reduced length of drug exposure, the addition of TFP did not produce a cytotoxic effect. In contrast to these results with P388/S cells, cytotoxic effects with VLB and VCR in the absence or presence of 2 and 4 μM TFP were observed in P388/DOX cells, only after 12 hr of drug treatment, and there was a 2- to 10-fold enhancement in cytotoxic effects due to TFP. In comparative studies, verapamil was found to be as effective as TFP in enhancing cytotoxic effects of VLB and VCR in P388/S and P388/DOX cells, suggesting a similar mechanism of action for the two agents in augmenting cell kill with vinca-alkaloids (unpublished results).

Based on cellular pharmacokinetic studies, the obvious rationale for using calmodulin inhibitors or calcium antagonists to overcome cellular resistance to vinca alkaloids would be to enhance cellular drug levels. Although results from this study, demonstrating an enhancement in VLB accumulation in P388/S and P388/DOX cells in the presence of TFP, are similar to the observations of Tsuruo *et al.* who studied the effect of calcium antagonists on accumulation of VCR in parent-sensitive and vincristine-resistant P388 mouse leukemia cells [20, 21], these data suggest a lack of correlation between cellular drug levels and cytotoxic response. Specifically, steady-state levels of VLB with or without TFP treatment are achieved in P388/S and P388/DOX cells in 1 hr, but cytotoxicity is evident in P388/S and P388/DOX cells only after treatment for 6 and 12 hr respectively. Furthermore, although there may be a relationship between cytotoxicity and cellular VCR levels in P388/S cells with and without TFP treatment, enhancement in cell kill due to TFP in VCR-treated P388/DOX cells occurs only after 12 hr, in spite of the fact that significant increases in cellular

VCR levels are reported to occur 1–3 hr after treatment [20, 21]. The effects of TFP in enhancing cellular retention of VLB were more pronounced with P388/DOX cells rather than in P388/S cells, and the role of TFP in increasing retention of VLB in P388/DOX cells appears to be by competition, since in cells treated with VLB and TFP during the 1-hr accumulation phase, and subsequently incubated in VLB-free medium, cellular retention of VLB was >4-fold higher in the presence than in the absence of TFP. Additionally, the data also suggest that the concentration of bound vinblastine is enhanced with TFP treatment, thus augmenting cellular retention of cytotoxic levels of vinblastine [11].

A comparison of our earlier data with anthracyclines and TFP in P388/S cells and P388/DOX cells [18, 23], and results from the present study with VCR and VLB, suggest that there are significant differences in the effects of TFP with these antitumor agents. In P388/S cells, TFP enhances only the cytotoxic effects but not accumulation or retention of DOX and DAU, in contrast to the marked increase in accumulation and cytotoxicity of VLB and VCR in the presence of TFP. In P388/DOX cells, however, although TFP significantly augments accumulation and cytotoxicity of DOX, DAU, VLB and VCR, enhanced cytotoxicity in the presence of TFP is evident at 1 hr of treatment with DAU or DOX, compared to 12 hr for VCR and VLB. The effects of TFP with the vinca alkaloids appear to be relatively non-specific, since significant enhancement in drug accumulation and cytotoxicity is observed with P388/S and P388/DOX cells. Furthermore, in P388/DOX cells, similar to results with lipophilic anthracyclines, e.g. *N*-trifluoroacetyladriamycin, AD41 [23], there appears to be no relationship between cellular vinca alkaloid levels and cytotoxicity, suggesting that, in addition to the requirement for threshold drug levels and cells to be in a sensitive phase of the cell cycle for maximal cell kill, other mechanisms may be involved in the cellular expression of vinca-alkaloid resistance.

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REFERENCES

1. W. A. Creasey, in *Cancer and Chemotherapy* (Eds. S. T. Crooke and A. W. Prestayko), Vol. III, p. 79. Academic Press, New York (1981).
2. R. A. Bender and B. A. Chabner, in *Pharmacologic Principles of Cancer Treatment* (Ed. B. Chabner), p. 256. W. B. Saunders, Philadelphia (1982).
3. P. W. Gout, R. L. Noble, N. Bruchovsky and C. T. Beer, *Int. J. Cancer* **34**, 245 (1984).
4. P. J. Ferguson, J. R. Phillips, M. Selner and C. E. Cass, *Cancer Res.* **44**, 3307 (1984).
5. J. A. Houghton, L. G. Williams, P. M. Torrance and P. J. Houghton, *Cancer Res.* **44**, 582 (1984).
6. M. Lane, *Fedn Proc.* **38**, 103 (1979).
7. K. Dano, *Cancer Chemother. Rep.* **56**, 701 (1972).
8. K. Dano, *Acta path. microbiol. scand.* Suppl. 256, 11 (1976).
9. R. K. Johnson, M. P. Chitnis, W. M. Embrey and E. B. Gregory, *Cancer Treat. Rep.* **62**, 1535 (1978).
10. L. J. Wilkoff and E. A. Dulmadge, *J. natn. Cancer Inst.* **61**, 1521 (1978).
11. W. T. Beck, *Adv. Enzyme Regulat.* **22**, 207 (1984).
12. M. Inaba, H. Kobayahsi, Y. Sakurai and R. K. Johnson, *Cancer Res.* **39**, 2200 (1979).
13. T. Skovsgaard, *Cancer Res.* **38**, 4722 (1978).
14. T. Skovsgaard, *Cancer Res.* **38**, 1785 (1978).
15. N. Kartner, M. Shales, J. R. Riordan and V. Ling, *Cancer Res.* **43**, 4413 (1983).
16. M. Inaba and R. K. Johnson, *Biochem. Pharmac.* **27**, 2123 (1978).
17. H. Riehm and J. L. Biedler, *Cancer Res.* **32**, 1195 (1972).
18. R. Ganapathi and D. Grabowski, *Cancer Res.* **43**, 3696 (1983).
19. R. Ganapathi, D. Grabowski, R. Turinic and R. Valenzuela, *Eur. J. Cancer clin. Oncol.* **20**, 799 (1984).
20. T. Tsuruo, H. Iida, S. Tsukagoshi and Y. Sakaurai, *Cancer Res.* **42**, 4730 (1982).
21. T. Tsuruo, H. Iida, S. Tsukagoshi and Y. Sakurai, *Cancer Res.* **41**, 1967 (1981).
22. T. Tsuruo, H. Iida, M. Nojiri, S. Tsukagoshi and Y. Sakurai, *Cancer Res.* **43**, 2905 (1983).
23. R. Ganapathi, D. Grabowski, W. Rouse and F. Riegler, *Cancer Res.* **44**, 5056 (1984).
24. R. Ganapathi, W. Reiter and A. Krishan, *J. natn. Cancer Inst.* **68**, 1027 (1982).
25. T. Tsuruo, H. Iida, H. Kawabata, S. Tsukagoshi and Y. Sakurai, *Cancer Res.* **44**, 5095 (1984).
26. L. J. Wilkoff and E. A. Dulmadge, *J. natn. Cancer Inst.* **68**, 1023 (1982).
27. D. V. Jackson and R. A. Bender, *Cancer Res.* **39**, 4346 (1979).