Cyclosporin A Reverses Vincristine and Daunorubicin Resistance in Acute Lymphatic Leukemia In Vitro

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Abstract

The development of drug resistance by tumor cells is a major obstacle to the cure of human malignancy. Cyclosporin A (CsA) completely reverses primary resistance to vincristine and cross resistance to daunorubicin in a pleiotropic drug-resistant subline of human T cell acute lymphatic leukemia. This subline is over 50-fold resistant to vincristine and fivefold resistant to daunorubicin. CsA has little effect on vincristine or daunorubicin activity in drug-sensitive parental leukemia and corrects daunorubicin resistance without altering cellular daunorubicin accumulation.

Introduction

There has been recent interest in the ability of calcium channel blocking agents and calmodulin inhibitors to enhance the responses of drug-resistant tumor cells to vinca alkaloids and to anthracycline antibiotics (1–3). The mechanism of this effect relates in part to the ability of these agents to inhibit the enhanced active efflux of vinca alkaloids and anthracycline antibiotics by resistant cells, resulting in increased cellular drug retention (1, 4). Since the acquisition of equimolar concentrations of anthracyclines by anthracycline-resistant compared with anthracycline-sensitive tumor cells fails to restore equivalent cytotoxic drug effect to these cells, the mechanism by which calcium channel blocking agents and calmodulin inhibitors restore drug sensitivity must extend beyond drug retention (5, 6). We now report that cyclosporin A (CsA)[1] reverses vincristine (VCR) resistance in VCR-resistant human T cell acute lymphatic leukemia (ALL) and corrects the daunorubicin cross resistance of this leukemia subline. CsA has little effect on VCR or daunorubicin activity in drug-sensitive parental ALL, nor does it alter uptake or efflux of daunorubicin by daunorubicin-resistant ALL cells.

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1. *Abbreviations used in this paper:* ALL, acute lymphatic leukemia; CsA, cyclosporin A; ED_{50}, 50% effective dose; L_0, drug-sensitive tumor line; L_{100}, VCR-resistant subline; VCR, vincristine.

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Methods

A VCR-resistant subline of GM3639 cells (Human Genetic Mutant Cell Repository, Camden, NJ) was developed by continuous in vitro exposure of GM3639 (L_0) cells, initially to sublethal and then to progressively increased concentrations of vincristine as previously described (7). The drug-resistant subline (L_{100}), grown continuously in 100 nM VCR, shows primary resistance to vincristine and cross resistance to daunorubicin and to VP-16 (7). VCR sensitivity of L_0 and L_{100} cells is measured in a cytotoxicity assay, in which cell viability in increasing concentrations of VCR is compared with cell viability in the absence of VCR (7). Daunorubicin sensitivity of L_0 and L_{100} cells is measured by the ability of daunorubicin to inhibit thymidine incorporation into DNA in these cell lines as previously described; this method correlates well with responses of daunorubicin-sensitive and daunorubicin-resistant Ehrlich ascites carcinoma in vivo (2). Uptake and efflux of [^{3}H]daunorubicin by these leukemic cell lines is measured by previously described methods (8). CsA was kindly provided in powder form by Dr. David Winter, Sandoz, Inc., East Hanover, NJ.

Results

Fig. 1 compares the effects of increasing concentrations of CsA on VCR cytotoxicity in drug-sensitive L_0 (*A*) and VCR-resistant L_{100} cells (*B*) and Fig. 2 compares the effects of increasing concentrations of CsA on daunorubicin inhibition of DNA synthesis in these same cell lines. Table I presents the statistical evaluation of three or more such experiments. L_{100} cells are over 50-fold resistant to VCR and are fivefold resistant to daunorubicin compared with L_0 cells. The VCR concentration required for cytotoxicity of 50% of the cell population (ED_{50}) assayed at 72 h for L_0 cells is 11.9±2.4 nM and for L_{100} cells, 701.7±91.7 nM. The concentration of daunorubicin required for 50% inhibition of thymidine incorporation into DNA for L_0 cells is 1.6±0.7 μg/ml and for L_{100} cells is 8.4±2.4 μg/ml. The addition of 3.3, 6.6, and 13.2 μg/ml CsA to cultures containing VCR or daunorubicin significantly reduces the respective ED_{50} values for L_{100} cells, the greatest concentration resulting in values characteristic of drug-sensitive parental leukemia.

Fig. 3 is representative of three similar experiments which compare the transport of [^{3}H]daunorubicin by L_0 cells and L_{100} cells. After 30 min of drug uptake, [^{3}H]daunorubicin efflux from L_0 and L_{100} cells in the presence and absence of CsA is compared. It can be seen that daunorubicin uptake by daunorubicin-resistant cells is only slightly impaired compared with daunorubicin-sensitive cells, and that the uptake of daunorubicin by daunorubicin-resistant cells is unaltered by CsA. Similar experiments carried out over 120 min also show only slightly greater

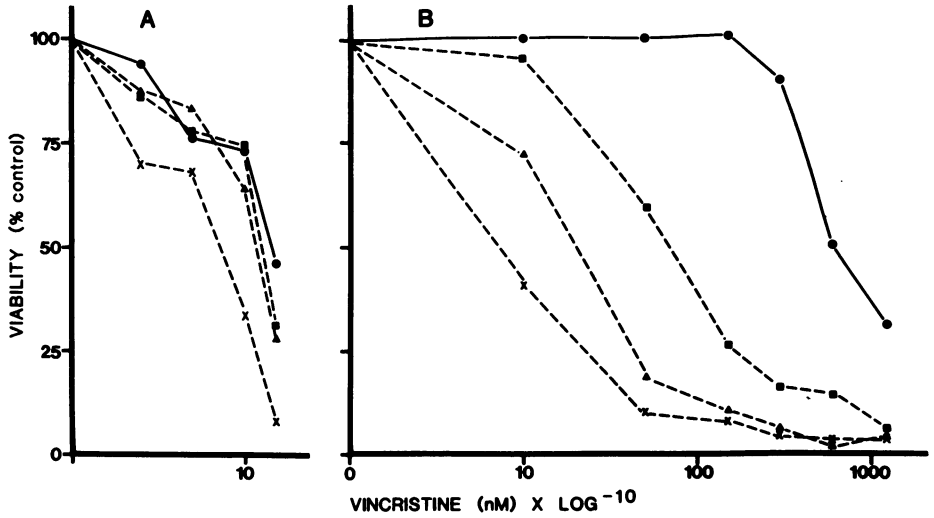

Figure 1. CsA effects on VCR ED_{50} values in drug-sensitive (*A*) and drug-resistant ALL (*B*). Cells are suspended in triplicate at 5×10^5 cells/ml of culture medium in cluster culture plates with VCR (0–1,200 nM) and CsA in a 3-d cytotoxic assay. Cell viability in increasing concentrations of drug is compared with cell viability in the absence of drug with CsA in a final concentration of 0 (— ● —), 3.3 μg/ml (--- ■ ---), 6.6 μg/ml (---▲---), and 13.2 μg/ml (---x---). After 3 d, equivalent aliquots are taken from each triplicate culture and counted in duplicate with a hemocytometer, and cell viability is determined by means of trypan blue dye exclusion. In this system, the range between duplicate counts is 10%. Each data point reflects the average of these counts divided by counts from control cultures without drug, and is expressed as percentage viability. The ED_{50} is defined as that concentration of drug resulting in 50% viable cells at 3 d compared with cells maintained without VCR.

[^{3}H]daunorubicin uptake by drug-sensitive vs. drug-resistant cells. The current series of experiments shows no effect of CsA on daunorubicin efflux from L_{100} cells. Fig. 3 demonstrates equivalent loss of [^{3}H]daunorubicin from L_{100} cells in the presence and absence of 13.2 μg/ml CsA.

Discussion

Our experiments show that CsA completely reverses primary resistance to VCR and cross resistance to daunorubicin in drug-resistant ALL. These effects are not limited to neoplastic human lymphoid cells since we have noted similar effects of CsA in daunorubicin-resistant Ehrlich ascites carcinoma in vitro and complete reversal of daunorubicin resistance by CsA in vivo (unpublished observations). In comparison to L_{100} cells, Ehrlich ascites carcinoma cells are resistant to daunorubicin because of prolonged direct exposure to daunorubicin in vivo (2). The daunorubicin-resistant Ehrlich ascites carcinoma subline is similar to the L_{100} subline since it also shows only slightly impaired uptake of daunorubicin compared with daunorubicin-sensitive parental tumor (8). Our current experiments also show that CsA fails to effect daunorubicin uptake or efflux by L_{100} cells, indicating that the restoration of daunorubicin responsiveness by CsA must depend on mechanisms independent of modified daunorubicin transport.

The CsA-restorative mechanism is unclear but may relate to calmodulin inhibition. It has recently been reported that human ALL cells have a 10-fold greater concentration of calmodulin than normal peripheral blood lymphocytes and that CsA is a potent inhibitor of T lymphocyte calmodulin (9, 10).

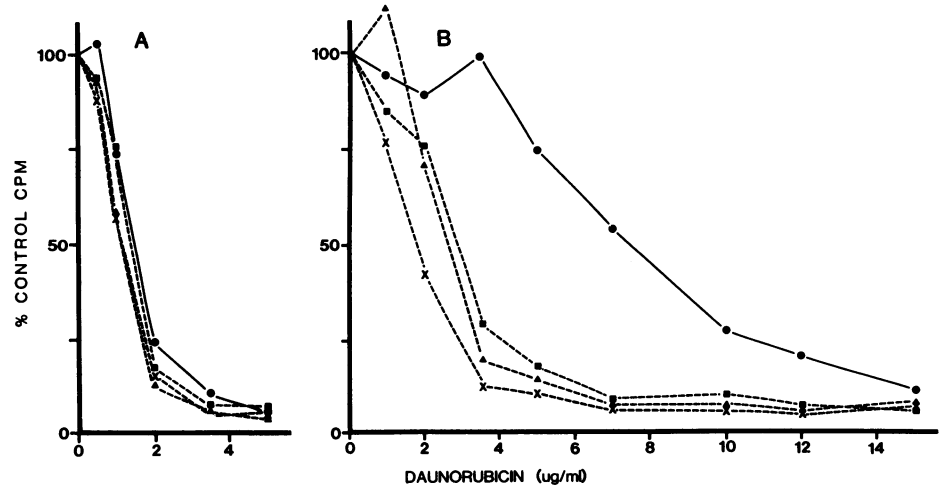

Figure 2. CsA effects on daunorubicin ED_{50} values in drug-sensitive (*A*) and drug-resistant ALL (*B*). Cells were counted on a hemocytometer using dye exclusion, washed, and resuspended in RPMI 1640 at a concentration of 1.6×10^6/ml. Cell aliquots of 1.6 ml were incubated with 0.2 ml daunorubicin HCL (final concentration 0–15 μg/ml) for 1 h at 37°C, washed twice, and resuspended in 1.8 ml RPMI and 0.2 ml cyclosporin A at a final concentration of 0 (— ● —), 3.3 μg/ml (--- ■ ---), 6.6 μg/ml (---▲---), and 13.2 μg/ml (---x---). Triplicate 180-μl aliquots were placed into microtiter plates and incubated with 20 μl [^{3}H]thymidine (specific activity 24 Ci/mmol, final concentration 1 μCi/ml) for 1 h at 37°C and 5% CO_2. The filters were dried and counted in a PPO/POPOP/toluene liquid scintillation system. Values are plotted as percent inhibition of [^{3}H]thymidine incorporation compared with control in the absence of daunorubicin.

Table I. CsA Alteration of VCR and Daunorubicin ED_{50} Values

Cell line	CsA	VCR		Daunorubicin	
		ED_{50}±SD	*P*	ED_{50}±SD	*P*
	μg/ml	*nM*		*μg/ml*	
(*A*) L_0	—	11.9±2.4	—	1.6±0.7	—
(*B*) L_{100}	—	701.7±91.7	<0.01 vs. (*A*)	8.4±2.4	<0.01 vs. (*A*)
(*C*) L_{100}	3.3	183.0±87.7	<0.05 vs. (*B*), >0.1 vs. (*A*)	4.9±1.4	>0.05 vs. (*B*), (*A*)
(*D*) L_{100}	6.6	43.3±29.2	<0.02 vs. (*B*), >0.1 vs. (*A*)	3.4±0.6	<0.01 vs. (*B*), <0.02 vs. (*A*)
(*E*) L_{100}	13.2	13.8±10.6	<0.01 vs. (*B*), >0.01 vs. (*A*)	2.3±0.5	<0.01 vs. (*B*), >0.1 vs. (*A*)

However, in the cell lines studied, anthracycline and velban-sensitive and -resistant ALL cells have the same calmodulin content, an observation which fails to explain the selective action of CsA in daunorubicin-resistant ALL (11, 12). The studies of LeGrue et al. (13) raise the possibility that CsA might function in the plasma membrane in a manner similar to that of lipid soluble anesthetics by increasing lipid fluidity and uncoupling electrochemical action potentials. Enhanced drug retention alone cannot account for the effects of calcium channel blocking drugs and calmodulin inhibitors on vinca alkaloid and anthracycline antibiotics in pleiotropic drug-resistant cells (5, 6). It has therefore been suggested that these agents, and it now appears that CsA, may promote favorable chemotherapeutic drug interactions at the membrane level or enhance intracellular drug binding (5, 12).

The clinical use of calcium channel blocking agents has been limited by high concentration requirements (14). However, since large doses of CsA have been administered to man with reasonable safety, and concentrations approaching those that are required for restoration of VCR and daunorubicin responses in ALL in vitro are achievable in vivo, CsA may prove to be a useful agent in the treatment of vinca alkaloid and anthracycline antibiotic-resistant neoplasia (15–17).

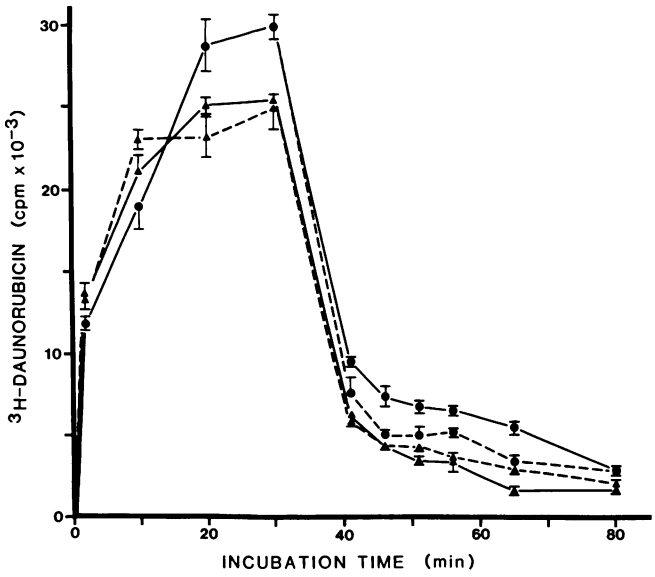

Figure 3. [^{3}H]daunorubicin uptake and efflux of sensitive (●) and resistant (▲) ALL cells. Cells were counted, washed, and resuspended at 22.5 × 10^5 cells/ml RPMI 1640 without (——) or with (- - -) 13.2 μg/ml cyclosporin A and [^{3}H]daunorubicin (specific activity 1.5–2.5 Ci/mmol, final concentration 5 μg/ml daunorubicin and 5 μCi/ml). Immediately and at designated time points, triplicate aliquots of 200 μl (at 37°C) were placed in microtiter plate wells and harvested onto glass fiber filters using a titertek multiple sample harvester. The filters were dried and counted in a PPO/POPOP/toluene liquid scintillation system. Efflux at 37°C was measured after 30 min of drug uptake. Aliquots were removed from the uptake suspension, centrifuged, washed, and resuspended in same volume of RPMI 1640. Triplicate 200-μl samples were taken immediately and at indicated time points and processed as above.

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