Biochemical Pharmacology, Vol. 35, No. 7, pp. 1087–1090, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

EFFECTS OF CYTOCHALASINS AND COLCHICINE ON THE ACCUMULATION AND RETENTION OF DAUNOMYCIN AND VINCRISTINE IN DRUG RESISTANT TUMOR CELLS

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(*Received* 14 *May* 1985; *accepted* 15 *October* 1985)

Abstract—Cytochalasin B and D enhanced vincristine (VCR) and daunomycin (DAU) accumulation in tumor cells, especially in VCR- and DAU-resistant cell lines. The effect of cytochalasin B, and to a lesser extent cytochalasin D, was almost equivalent to that observed for verapamil, a calcium channel blocker which has been reported to enhance drug accumulation in tumor cells. Cytochalasin B was most effective in VCR- and DAU-sensitive cells; however, the effect in resistant cells was less than that observed for verapamil, suggesting a different mode of action between these drugs in sensitive and resistant cells. Enhanced accumulation of VCR and DAU by cytochalasins was mediated by the inhibition of outward transport of VCR and DAU from tumor cells. Colchicine had no effect on VCR and DAU accumulation. Cytochalasins, especially cytochalasin D is a specific inhibitor of microfilament assembly in cells. These results indicate that the cellular microfilament system plays a prominent role in drug transport of tumor cells, and that an intact microtubular system is less involved.

Tumor cells which acquire resistance to vincristine (VCR) and adriamycin (ADM) actively transport these antitumor agents to the outside [1–4]. This results in a relatively low intracellular level of drug and thus to low cytotoxicity. We have observed that calcium channel blockers inhibit the efflux of VCR and ADM from tumor cells. Moreover, tumor cells initially resistant to VCR or ADM that have been treated with calcium channel blockers become sensitive to these agents [4–10].

The mechanism(s) of increased drug efflux of resistant tumor cells, and the reversal of this process by calcium channel blockers are not known. Presumably the plasma membrane and the subplasmalemmal cytoskeletal structure are intricately associated with these mechanisms. In order to examine this possibility, we have examined the effect of drugs which interact with cytoskeletal components. Colchicine binds to tubulin and inhibits its polymerization [11]. Cytochalasin B inhibits actin polymerization [12, 13]. In addition to this action, cytochalasin B binds to the cell membrane (Site I binding) and inhibits glucose transport [14–16]. Cytochalasin D inhibits actin polymerization, however, the compound neither binds at Site I of the plasma membrane, nor inhibits glucose transport activity [15–17].

We found colchicine had no effect on VCR and daunomycin (DAU) accumulation in resistant tumor cells; however, cytochalasin B and, to a lesser extent, cytochalasin D had a potent effect as has been observed previously for calcium channel blockers [4–10].

MATERIALS AND METHODS

Drugs. VCR, ADM and DAU were obtained from Shionogi and Co. (Osaka, Japan), Kyowa Hakko Kogyo Co. (Tokyo, Japan) and Meiji Seika Co. Ltd. (Tokyo, Japan), respectively. [G-^{3}H]VCR sulfate (5.8 Ci/mmol) and [G-^{3}H]DAU (4.34 Ci/mmol) were purchased from the Radiochemical Center (Amersham, U.K.). Cytochalasin B and D and colchicine were purchased from Nakarai Chemical Co. (Kyoto, Japan). Verapamil was provided by the Eisai Co. Ltd. (Tokyo, Japan).

Tumor cells. P388, P388/VCR and P388/ADM cell lines were supplied by the National Cancer Institute (NIH, Bethseda, MD) [4, 5, 10]. The human myelogenous leukemia K562 cell line [18] was provided by Dr Ezaki, and the K562/VCR cell line was established in this laboratory [19].

Cellular uptake of [^{3}H]VCR and [^{3}H]DAU in the presence of cytochalasins and colchicine. Cellular uptakes of VCR and DAU were measured in growth medium [4, 5]. In this experiment [^{3}H]DAU was used instead of [^{3}H]ADM because the latter compound is not available commercially. P388/VCR, P388/ADM and K562/VCR cells (2×10^5) in Falcon No. 2054 culture tubes containing 1 ml of growth medium with 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer were incubated at 37° in the presence of cytochalasins or colchicine with [^{3}H]VCR (10 nM; specific activity, 5.8 Ci/mmol) or [^{3}H]DAU (10 nM; specific activity, 4.34 Ci/mmol), and the amounts of intracellular VCR and DAU was measured at various times as described previously [4, 5, 20].

Extracellular transport of [^{3}H]VCR or [^{3}H]DAU and effect of cytochalasins and colchicine. VCR- and ADM-resistant tumor cells actively transport VCR and DAU to the outside of the cell and usually maintain very low intracellular levels of these drugs [1–4]. To obtain sufficiently high intracellular concentrations of drugs, we initially loaded P388/VCR, K562/VCR and P388/ADM cells with 33 nM [^{3}H]-

VCR and 33 nM [^{3}H]DAU in glucose-free growth medium supplemented with 10% dialyzed fetal bovine serum (Grand Island Biological Co.) in the presence of 10 mM sodium azide for 30 min as described previously [2–4, 20]. Then each cell suspension was centrifuged (80 g; 10 min; 5°) and the precipitated cells (2×10^5) were resuspended (more than 98% of the cells excluded trypan blue) in 1 ml of growth medium containing 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer and incubated at 37° in the absence or presence of cytochalasins and colchicine. At the indicated times, the intracellular [^{3}H]VCR and [^{3}H]DAU levels were determined as described previously [4, 5].

RESULTS

Viability of the cells treated with cytochalasins and colchicine

The viability, as determined by trypan blue dye exclusion, of P388/VCR cells after treatment of the cells with cytochalasins and colchicine for 2 hr at 37° in the growth medium is shown in Table 1. Cytochalasins did not induce significant cytotoxicity at 60 μM. At 200 μM, a slight cytotoxicity was observed, especially for cytochalasin B. Colchicine did not induce cytotoxicity at 0.5 μM. At 1.5 or 5 μM, 13–15% cytotoxicity occurred with colchicine. Viabilities of P388, P388/ADM, K562 and K562/VCR after treatment of the cells with cytochalasins and colchicine were usually higher than that obtained for P388/VCR cells (data are not shown).

Based on these results, we used the cytochalasins at 20 μM and colchicine at 0.5 μM for transport experiments, where no significant cytotoxicity occurred, but where the function of actin and tubulin can be inhibited [11–17].

Table 1. Viability of P388/VCR cells after treatment the cells with cytochalasins and colchicine*

Drug and concentration (μM)		% of dye excluded cells (% of control)
Cytochalasin B	0	89 ± 6.8 (100)
	20	86 ± 3.6 (96)
	60	86 ± 5.6 (96)
	200	81 ± 4.8 (91)
Cytochalasin D	0	90 ± 2.3 (100)
	20	88 ± 1.4 (98)
	60	92 ± 2.6 (103)
	200	87 ± 1.3 (97)
Colchicine	0	89 ± 1.3 (100)
	0.5	87 ± 2.1 (98)
	1.5	78 ± 4.8 (87)
	5.0	76 ± 4.5 (85)

* P388/VCR cells were collected by centrifugation and were treated with drug for 2 hr at 37° in growth medium at cell density 2×10^5/ml. Cells were collected by centrifugation and suspended at 1×10^6/ml of 0.1% trypan blue solution. At least 10^3 cells were counted and % of dye excluded cells was calculated. The values are mean ± S.D. of 3 independent determinations.

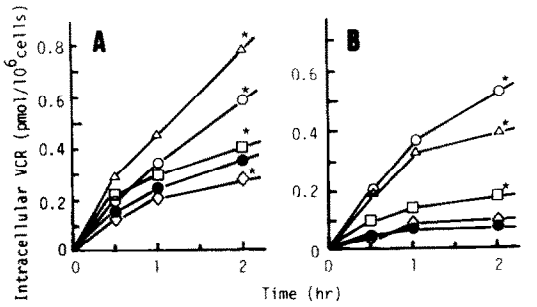

Fig. 1. Effects of cytochalasins, colchicine and verapamil on cellular accumulation of [^{3}H]VCR in P388 and P388/VCR cells. P388 (A) or P388/VCR (B) cells were incubated in growth medium with 10 nM [^{3}H]VCR in the absence (●) or presence of cytochalasin B at 20 μM (△), cytochalasin D at 20 μM (□), colchicine at 0.5 μM (◇) and verapamil at 2.2 μM (○). Each point is the mean of triplicate determinations, and S.D. is within 7.1% of the value of each point. The results at 2 hr were analyzed for significance (*$P < 0.05$) by means of the t-test as compared to the results for the control experiment (●).

Effects of cytochalasins and colchicine on drug accumulation

The time course of VCR uptake by P388 and P388/VCR cells and the effect of cytochalasins, colchicine and verapamil are shown in Fig. 1. In P388 cells, cytochalasin B stimulated the accumulation of VCR, where an approximately 2-fold enhancement occurred as compared to the control cells. Cytochalasin D was less effective and only 15–20% enhancement was observed. Verapamil moderately enhanced the VCR accumulation, however, the effect was less than that observed for cytochalasin B. Colchicine had no stimulating effect on VCR accumulation. In P388/VCR cells, the most efficient enhancement of VCR accumulation occurred with verapamil and resulted in approximately 8-fold

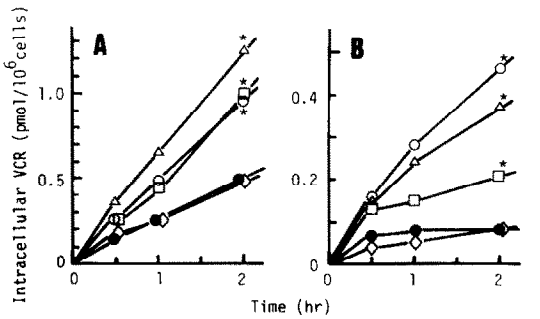

Fig. 2. Effects of cytochalasins, colchicine and verapamil on cellular accumulation of [^{3}H]VCR in K562 and K562/VCR cells. K562 (A) or K562/VCR (B) cells were incubated in growth medium with 10 nM [^{3}H]VCR in the absence (●) or presence of cytochalasin B at 20 μM (△), cytochalasin D at 20 μM (□), colchicine at 0.5 μM (◇) and verapamil at 2.2 μM (○). Each point is the mean of triplicate determinations, and SD is within 9.3% of the value of each point. The results at 2 hr were analyzed for significance (*$P < 0.05$) by means of the t-test as compared to the results for the control experiment (●).

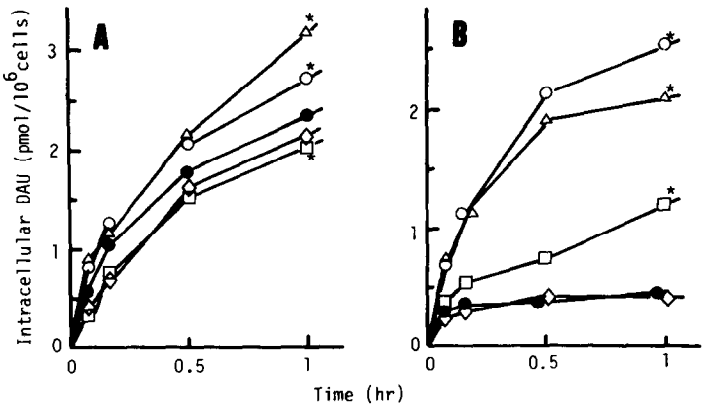

Fig. 3. Effects of cytochalasins, colchicine and verapamil on cellular accumulation of [^{3}H]DAU in P388 and P388/ADM cells. P388 (A) or P388/ADM (B) cells were incubated in growth medium with 10 nM [^{3}H]DAU in the absence (●) or presence of cytochalasin B at 20 μM (△), cytochalasin D at 20 μM (□), colchicine at 0.5 μM (◇) and verapamil at 2.2 μM (○). Each point is the mean of triplicate determinations, and SD is within 11.2% of the value of each point. The results at 1 hr were analyzed for significance (*$P < 0.05$) by means of the *t*-test as compared to the results for the control experiment (●).

enhancement. The effect of cytochalasin B was also prominent, however, the effect was less than that observed for verapamil. Cytochalasin D enhanced the VCR accumulation by 2–2.5-fold. Colchicine, again, showed only a marginal effect on drug accumulation. In human myelogenous leukemia K562 and its vincristine-resistant subline, a similar phenomenon was observed (Fig. 2). Briefly, cytochalasin B was most effective in sensitive cells and verapamil possessed the highest effect on resistant cells. These results indicate that the drug transport mechanisms of the resistant tumors cells might differ from those of sensitive cells, and that cytochalasin and verapamil might act on different sites of the drug transport mechanism(s) of these resistant tumor lines.

The effects of these drugs on DAU accumulation in sensitive and adriamycin-resistant P388 leukemia cells is shown in Fig. 3. In this experiment, the drugs did not induce such an enhanced accumulation as observed for VCR in sensitive cells. Only 15% of enhancement was observed for cytochalasin B and verapamil. In resistant cells, verapamil and cytochalasin induced a highly enhanced (5- to 6-fold) accumulation of DAU. Cytochalasin D also showed significant effect but colchicine again had no effect on DAU accumulation.

The enhanced accumulation of VCR and DAU on

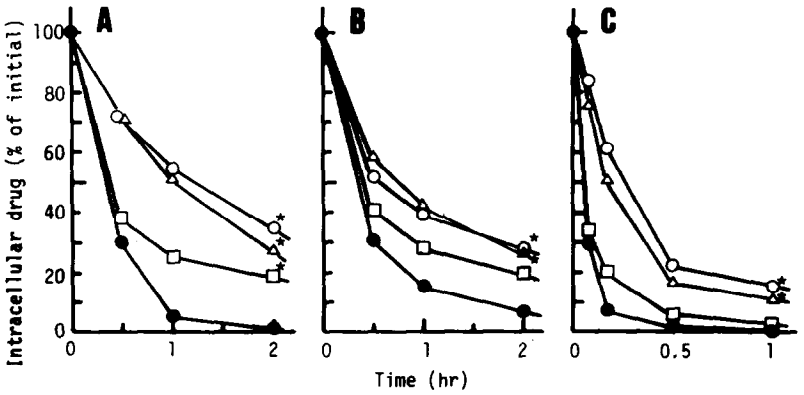

Fig. 4. Effects of cytochalasins, colchicine and verapamil on the efflux of [^{3}H]VCR and [^{3}H]DAU from the cells. P388/VCR (A), K562/VCR (B), or P388/ADM (C) cells were incubated in growth medium with 10 mM sodium azide for 10 min. Then 33 nM [^{3}H]VCR (A and B) or [^{3}H]DAU (C) was added, and incubated for 30 min. Then, each cell suspension was centrifuged, and precipitated cells were resuspended and incubated at 37° in growth medium in the absence (●) or presence of cytochalasin B at 20 μM (△), cytochalasin D at 20 μM (□), and verapamil at 2.2 μM (○). Each point is the mean of triplicate determinations, and SD is within 10.8% of the value of each point. The results at 2 hr (A and B) and 1 hr (C) were analyzed for significance (*$P < 0.05$) by means of the *t*-test as compared to the results for the control experiment (●).

resistant tumor cells by these drugs was mediated, at least in part, by the inhibition of VCR and DAU efflux from resistant tumor cells (Fig. 4). Verapamil and cytochalasin B possessed the most prominent inhibitory effect on drug efflux. Cytochalasin D showed a lesser effect on drug efflux.

DISCUSSION

The cytoskeletal system, which includes microtubules and microfilaments, is intimately involved in cell shape and movement. Among the inhibitors which interfere with the function and structure of the cytoskeletal network, cytochalasins, especially cytochalasin B, have been reported to inhibit a wide variety of cell functions which include movement, phagocytosis, pinocytosis, secretion of hormones and the inhibition of morphogenesis [12]. The effect of cytochalasins is due to an inhibition of microfilament function [12]. We observed here that cytochalasins, especially cytochalasin B, efficiently inhibited the outward transport of VCR and DAU from tumor cells resistant to these drugs. Subsequently, a great enhancement of cellular accumulation of these drugs in resistant tumor cells is attained. These results indicate that the function of microfilaments is involved in the outward transport of antitumor agents from tumor cells. We observed that cytochalasin B always possessed a stronger effect than cytochalasin D. Cytochalasin B has been reported to inhibit the glucose transport [14–16], in addition to the inhibition of the function of microfilament [12, 13]. However, cytochalasin D is rather a specific inhibitor of microfilament function, and has no inhibitory effect on glucose transport [15–17]. Outward transport of antitumor agents in drug-resistant tumor cells is dependent on energy and thus the process is inhibited by glucose starvation or by energy uncouplers [1–3, 5, 9, 19]. The stronger effect of cytochalasin B can be obtained by the starvation of glucose in the cells which could be induced by the inhibition of glucose transport by cytochalasin B. Verapamil, a calcium channel blocker, also possessed strong inhibitory action on outward drug transport. The mechanism of action of verapamil is not necessarily identical to that of cytochalasins; however, the effects of verapamil on microfilament function as well as on energy-producing systems, including glucose transport, are worthy of examination.

Acknowledgements—We thank Dr W. Fogler, M. D. Anderson Hospital and Tumor Institute for critical reading of the manuscript.

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