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RESTORED IN VITRO SENSITIVITY OF ADRIAMYCIN- AND VINCRISTINE-RESISTANT P388 LEUKEMIA WITH RESERPINE

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Cross-resistance between anthracycline antibiotics and vinka alkaloids has been elucidated in a number of sublines of mouse malignant cell lines resistant to these agents (1-7). Furthermore, from studies on the mechanisms of the resistance, evidence was obtained that these resistant cells were endowed with an active efflux system common to dissimilar classes of these agents (8-12). In an attempt to clarify the more detailed biochemical mechanisms of this active efflux system, a survey was conducted to search, among various membrane active agents, for substance(s) which potentiate the sensitivity of these resistant cells to such kinds of anticancer agents using the in vitro cell culture technique.

MATERIALS AND METHODS

Adriamycin(ADR) and vincristine(VCR)-resistant sublines of P388 leukemia (P388/ADR, P388/VCR) were established by in vivo sequential treatment (13-14). ADR, VCR and reserpine were purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Shionogi & Co., Ltd., Osaka, and Nakarai Chemicals, Ltd., Kyoto, Japan, respectively. Reserpine was dissolved in dimethylsulfoxide(DMSO) and then diluted with culture medium. DMSO concentration was 0.005% at the highest. As for radioactive drugs, [G-^{3}H]-VCR(2.85 Ci/m mol) was purchased from Radio Chemical Centre, Amersham, England and [^{14}C]-ADR (7.3 mCi/m mol) was provided by Stanford Research Institute, Stanford, Cal., U. S. A.

Primary cell culture of P388 leukemia and the measurement of growth inhibitory effect and cellular uptake of radioactive drugs have been described elsewhere (10).

RESULTS AND DISCUSSION

Among a number of membrane active agents, reserpine was found to have a potent synergistic effect on growth inhibition with ADR and VCR of the resistant cells. The differences in sensitivity to ADR and VCR between the sensitive and each of the resistant sublines were approximately 100 and 300,

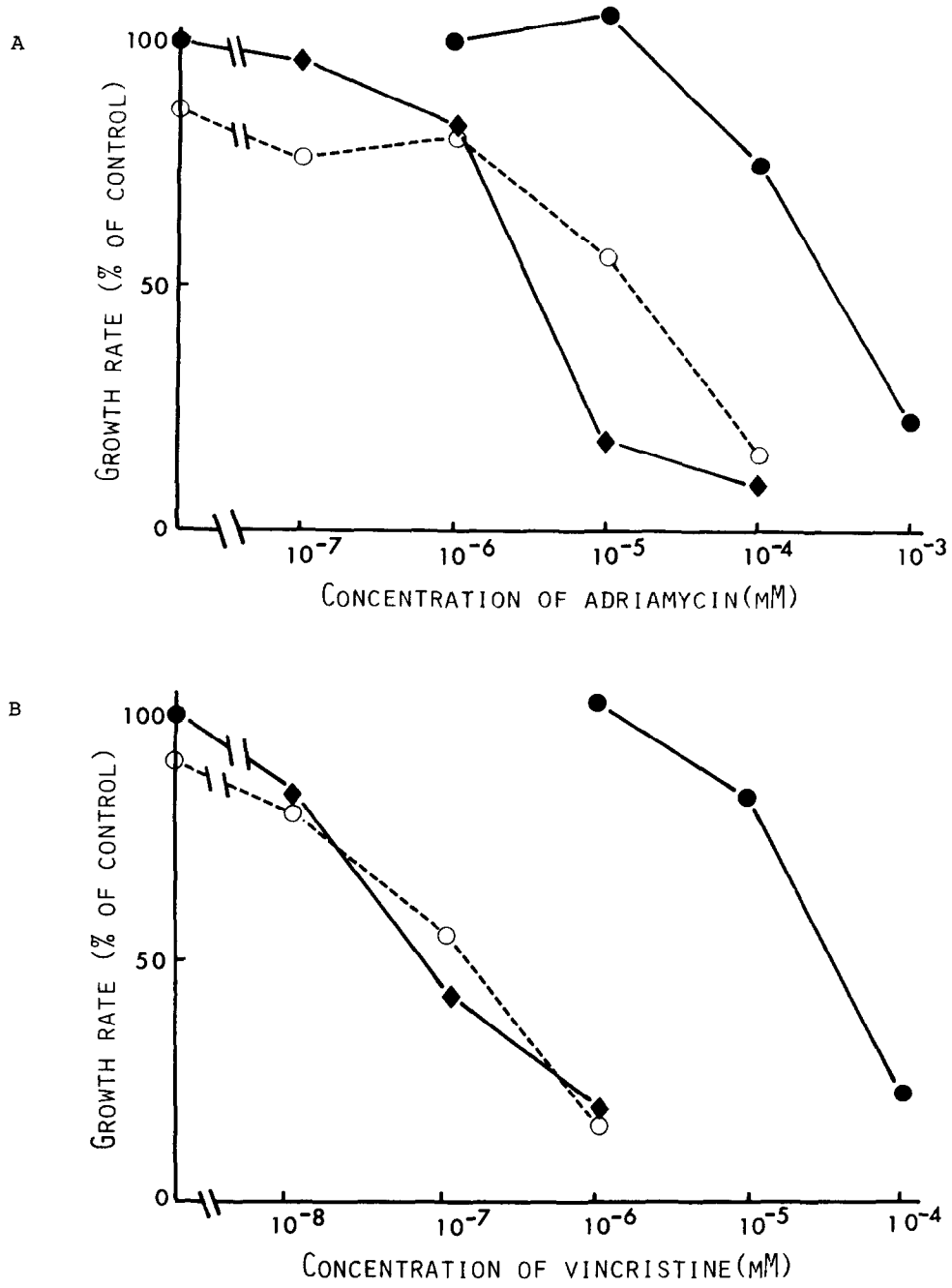

Fig. 1. Effects of reserpine on in vitro sensitivity of adriamycin- and vincristine-resistant sublines of P388 leukemia to each of these antitumor agents. Cells of P388/ADR(A) and P388/VCR(B) were cultured with varying concentrations of ADR and VCR, respectively, in the presence (○---○) or absence (●——●) of 10^{-3} mM reserpine for 48 hr. ◆——◆ shows the growth inhibition curves with ADR(A) or VCR(B) of the sensitive cells.

respectively, when they were expressed as ratios of IC_{50} (drug concentration for 50% growth inhibition) for the resistant line to the sensitive one. With P388/ADR, this difference became only 5 in the presence of 10^{-3} mM reserpine, while the sensitivity of P388/VCR to VCR was restored to almost that of the sensitive P388, as shown in Fig. 1. On the other hand, reserpine did not exert a profound effect on the sensitivity of the cells sensitive to these antitumor agents.

The reason why reserpine showed such a potent effect on the sensitivity of these resistant cells remains obscure. However, from the viewpoint of the biochemical mechanism reported with resistance to ADR and VCR (8-12), it is very probable that reserpine influenced the uptake of these antitumor agents by the resistant cells. The preliminary results shown in Table 1 demonstrate that uptake of ADR and VCR was significantly increased with reserpine exclusively by the resistant cells.

Table 1. Effects of reserpine on uptake of adriamycin and vincristine by sensitive and adriamycin-, vincristine-resistant P388

Radioactive drugs	Reserpine	Cellular uptake (p mol/10^6 cells)		
		P388/S	P388/ADR	P388/VCR
adriamycin	-	57.4	40.3	37.5
	+	54.0	58.7	69.0
vincristine	-	0.061	0.038	0.034
	+	0.056	0.064	0.066

Cells were pre-incubated in Hank's balanced salt solution with or without 10^{-2} mM reserpine for 10 min at 37°C and then small quantities of [^{14}C]-ADR or [^{3}H]-VCR solution were added to adjust to a final concentration of 10^{-3} and 10^{-5} mM, respectively. After 30 min incubation at 37°C, the radioactivity of the washed cells was counted in a Beckman Model LS-355 liquid scintillation counter.

It is known that reserpine induces a significant decrease in the calcium content of vascular, cardiac and brain tissues (15,16). Recently, from in vitro studies using spin-labeled artificial membrane, it has become evident that reserpine caused the release of calcium ions previously bound to the membrane lipids (17). On the other hand, we observed that chlorpromazine (18) and dibucaine (19,20), which have been reported to be inhibitors of Ca^{++}-binding to phospholipids, also enhanced the uptake of ADR and VCR by these resistant cells (data not shown). These facts strongly suggest that the active efflux system for ADR and VCR is profoundly correlated with some Ca^{++}-dependent enzymes or biological systems such as cyclic-AMP-related enzymes, Ca^{++}, Mg^{++}-ATPase and so on. Further biochemical studies on this novel transport system are in progress.

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