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Short communication

Adriamycin-induced inhibition of arachidonate incorporation into phospholipids of mastocytoma P815 cells

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Summary. Adriamycin inhibited [1-^{14}C]-arachidonic acid uptake by mastocytoma P815 cells in a dose-dependent manner (5–120 nM). The incorporation of [1-^{14}C]-arachidonic acid into phospholipids was decreased mainly into cardiolipin and phosphatidylcholine.

Introduction

The mechanism of the membranotoxic action of Adriamycin (ADR), a very effective antineoplastic agent, has not been clarified. Recent studies indicate that ADR alters such membrane properties as fluidity [6], permeability [2], and transport of ions [1] and induces the peroxidation of membrane lipids [5]. However, the structural and functional properties of cellular membranes depend on phospholipid metabolism, and phospholipid turnover may possibly be involved in ADR-induced tumor-cell toxicity. In this report we provide evidence that phospholipid reacylation can be modified by ADR.

Materials and methods

Cell cultures. Transformed murine mastocytoma P815 mast cells were transplanted i. p. weekly into DBA/2 mice. For in vitro assay, tumor cells were harvested from the abdominal cavity of tumor-bearing mice. Finally, the cells were suspended in 199 medium.

Assay of [1-^{14}C]-arachidonic acid uptake by cells and incorporation into phospholipids. For determination of [1-^{14}C]-arachidonic acid (AA) uptake, tumor cells (10^5) were incubated with 199 medium containing ADR (Farmitalia Carlo Erba, Italy) at concentrations of 120, 24, and 5 nM for 1 h at 37° C, and 0.05 μCi [1-^{14}C]-AA (Amersham, UK) was added for a 5-min period. After two washes with AA-free medium containing 0.1% bovine serum albumin, cellular radioactivity was counted in a Mark-3 scintillation counter (Tracor Analytic, USA). Study of AA incorporation (0.2 μCi) into cellular phospholipids was carried out under similar conditions. Phospholipids were extracted according to the method of Folch et al. [4] and separated by high-performance liquid chromatography (HPLC; Ultrachrom G Ti, LKB, Sweden) on a 4.6– ×250-mm TSK-Si-150 Ultropac column (particle size, 5 μm). Elution was carried out using 100% solvent A (hexane/2-propanol; 80:20, v/v) for 5 min followed by continuous gradient from 100% solvent A to 100% solvent B (hexane/2-propanol/water; 39:52:9, by vol.) for 30 min; the flow rate was 1 ml/min. Fractions of the eluate were collected every 60 s for the measurement of radioactivity. Aliquots were taken for the determination of phospholipid phosphorus (Pi) by a micromethod [7].

Results and discussion

The dose-dependent effect of ADR on the AA uptake by P815 cells is presented in Table 1. Cells were pretreated for 1 h with 5, 24, or 120 nM ADR prior to their incubation with AA. Exposure of mastocytoma P815 cells to exogenous [1-^{14}C]-AA resulted in the incorporation of ^{14}C label into cardiolipin (CL), phosphatidylethanolamine, phosphatidylinositol, and phosphatidylcholine (PC; Fig. 1). As shown in the chromatograms, labeling of the tumor cells in the presence of ADR (120 nM) led to a reduction of [1-^{14}C]-AA incorporation mainly into CL.

Table 1. Effect of ADR on the uptake of [1-^{14}C]-AA by mastocytoma P815 cells

ADR in medium (nM)	Radioactivity (dpm/15^5 cells)	% Control
0	17,309 ± 2,391	100
120	8,452 ± 1,095*	48.8
24	11,486 ± 1,728*	66.3
5	13,610 ± 1,191	72.8

Cells were incubated with different concentrations of ADR for 1 h and then labelled with 0.05 μCi [1-^{14}C]-AA for 5 min. Data represent mean values ± SD for 8 experiments.
* P <0.01 as determined using Student's t-test

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Table 2. Effect of ADR on the incorporation of [1-^{14}C]-AA into various phospholipids of mastocytoma P815 cells

Lipids	Radioactivity (dpm/μg Pi)		
	Control	ADR (nM)	
		120	4
Total phospholipids	102,819 ± 12,754	71,138 ± 8,994	87,645 ± 1,098
Phosphatidylcholine	35,242 ± 4,320	22,793 ± 2,674*	33,850 ± 4,789
Cardiolipin	67,750 ± 6,468	37,045 ± 4,261*	41,637 ± 4,632*
Phosphatidylinositol	5,919 ± 936	4,527 ± 794	4,255 ± 934
Phosphatidylethanolamine	8,088 ± 965	6,772 ± 1,014	79,025 ± 892

Cells (1.5×10^6) were incubated with different concentrations of ADR for 1 h and then labelled with 0.2 μCi [1-^{14}C]-AA for 5 min. Lipids were extracted according to the method of Folch et al. [4] and separated by HPLC. Data represent mean values ± SD for 6 experiments.
* $P < 0.01$ as determined using Student's t-test

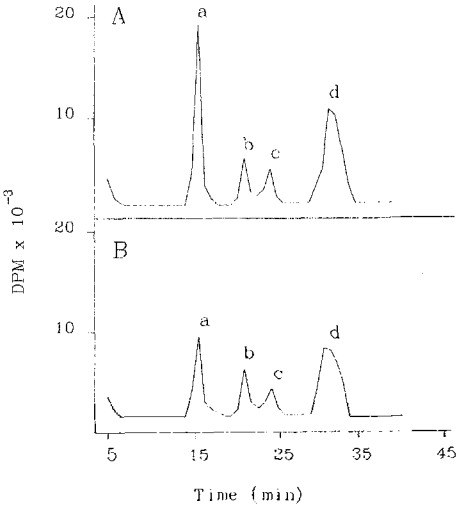

Fig. 1 A, B. Normal-phase HPLC profiles of [1-^{14}C]-arachidonate-containing phospholipids extracted from mastocytoma P815 cells **A** before and **B** after ADR treatment. *a*, Cardiolipin; *b*, phosphatidylethanolamine; *c*, phosphatidylinositol; *d*, phosphatidylcholine

Table 2 shows the incorporation of AA into various phospholipids of mastocytoma P815 cells. Preincubation of the cells with ADR (120 nM) for 1 h at 37°C prior to the addition of AA resulted in a decrease in the arachidonate incorporation mainly into CL and PC. In contrast, ADR did not significantly alter the incorporation of [1-^{14}C]-AA into phosphatidylethanolamine and phosphatidylinositol.

Our results clearly demonstrate the involvement of phospholipid reacylation in the effect of ADR on tumor cells during short-term incubation. In contrast, when myocardial cells were incubated with ADR, an inhibition of fatty-acid incorporation into cellular phospholipids was observed only at 20–24 h after drug exposure [3]. Thus, the mechanisms of ADR action differ in various kinds of cells.

Uptake of fatty acids into membrane phospholipids is catalyzed by fatty acyl coenzyme A acyltransferase. Unfortunately, studies devoted to ADR-induced alterations in this enzyme in tumor cells are lacking.

In summary, the present results allow us to conclude that the multiple mechanisms underlying the effect of ADR on tumor-cell membranes include phospholipid reacylation.

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