ACTIVATION OF PHOSPHOLIPASE HYDROLYSIS DURING OXIDATIVE CELL DAMAGE

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One of the integral manifestations of cell death resulting from the action of various toxic agents is a disturbance of the structural and functional organization of the cell membranes; a leading role in this process is played by disturbance of metabolism of the membrane phospholipids. It has been shown [9] that the resistance of tumor cells to killing by cytotoxic T lymphocytes does not correlate with synthesis of DNA, RNA, and protein or with total synthesis of the cell lipids, but does correlate with the synthesis and composition of cellular phospholipids, which as we know are the principal components of biological membranes. If the cytotoxic action of complement on a culture of guinea pig hepatoma is potentiated by adriamycin, the lysophosphatidylcholine (LPC) concentration is increased in various cell membranes [8]. Exogenous LPC and phospholipase A_2 (PLA_2), which hydrolyze phosphatidylcholine (PC) to free fatty acids and LPC, have a cytotoxic action on various cells [2, 10, 12], moreover, addition of PLA_2 inhibitors, namely bromphenacyl bromide, quinacrine, and mepacrine, reduces the sensitivity of cells to toxic agents [2, 6]. Phospholipase hydrolysis may perhaps be a universal mechanism that is realized during cell death, including during oxidative cell damage.

The aim of this investigation was to study activity of PLA_2 and different classes of phospholipids on mouse mastocytoma P815 cells exposed to the cytotoxic action of tert-butyl hydroperoxide (TBHP).

EXPERIMENTAL METHOD

Mastocytoma P815 cells were used in the experiments on the 7th day after transplantation intraperitoneally into DBA2 mice. Incorporation of [1-^{14}C]-arachidonic acid ("Amersham," England) into isolated cells (1 μCi/10^6 mastocytoma cells) was carried out at 37°C for 1 h, after which the cells were washed 3 times with medium 199 containing 0.1% albumin. To determine the release of labeled arachidonic acid into the aqueous phase the cells were sedimented at different time intervals after incubation with TBHP, the supernatant was transferred into scintillation flasks, and radioactivity was counted on a "Mark 3" liquid scintillation counter (Holland). Lipids were extracted from the cells by the method in [3]. Separation of phospholipids into classes was carried out by two-way thin-layer chromatography on silica gel Kiesegel 60 F_{254} ("Merck") in the microversion [14]. Lipid phosphorus was determined by the method of Vas'kovskii and co-workers [15]. The damaging action of TBHP on the plasma membrane of the cells was estimated by measuring the release of ^{3}H-uridine from previously labeled cells [1]. The results were subjected to statistical analysis by the nonparametric U test.

EXPERIMENTAL RESULTS

Preliminary studies of the cytotoxic action of TBHP on a population of mouse mastocytoma P815 cells showed that after incubation for 24 h the tumor cells died and this was accompanied by gross disturbances of cell membrane permeability, starting with TBHP in a concentration of $3 \cdot 10^{-10}$ M. Investigation of the time course of the membranotoxic damage to

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TABLE 1. Effect of TBHP on Content of Various Classes of Phospholipids in Mouse

Incubation time, min	Relative content,					
	sphingomyelin		phosphatidylserine		phosphatidic acid	
	experiment	control	experiment	control	experiment	control
0	5,20±1,08	5,07±1,34	5,65±1,23	4,12±1,27	2,14±0,91	3,87±1,01
30	8,89±1,53*	4,25±1,21	7,33±2,57*	2,41±1,24	9,05±2,17*	3,35±1,15
60	13,01±2,09*	6,10±1,48	8,45±2,39	6,52±2,15	11,12±2,72*	6,32±2,25
90	14,31±2,59*	5,83±1,04	12,89±2,21*	6,32±0,99	11,94±3,21*	6,46±1,45

Legend. Asterisk indicates significance of differences from control, $p < 0.05$.

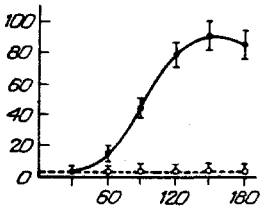

Fig. 1

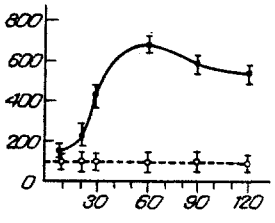

Fig. 2

Fig. 1. Changes in permeability of plasma membrane of P815 cells exposed to 20 μM TBHP. Continuous line — TBHP, broken line — control. Abscissa, duration of incubation (in min); ordinate, membranotoxicity (in % of control).

Fig. 2. Mobilization of [1-^{14}C]-arachidonic acid from P815 mastocytoma cells under the influence of 20 μM TBHP. Ordinate, mobilization of [1-^{14}C]arachidonic acid (in % of control). Remainder of legend as to Fig. 1.

P815 cells in the presence of $2 \cdot 10^{-5}$ M TBHP showed that permeability of the membrane for high-molecular-weight substances was increased starting with 60 min of incubation, and toward 180 min the destruction of the plasma membranes of the cells was complete (Fig. 1).

By contrast, mobilization of arachidonic acid from membrane phospholipids into the aqueous phase began to increase as a result of activation of PLA_2 as early as 20 min of exposure to the toxic action of TBHP, and by 60 min it reached its maximum (Fig. 2). Accumulation of LPC, another product of phospholipase hydrolysis, continued throughout the period of incubation of the P815 cells with 20 μM TBHP, and by 60 min its content in the experimental sample was 7 times greater than in the control (Table 1).

Thus toward the beginning of lysis of the cells (60 min) about 15% of LPC accumulated in their membranes, and this may perhaps be a critical factor in membrane stability, for later in the 60-90 min time interval 80% of the cells underwent lysis. The membrane-solubilizing activity of lysophospholipids has been repeatedly discussed in the literature in connection with their amphiphilicity [10]. For instance, the role of exogenous LPC in the onset of disturbances of membrane structure has been demonstrated for myelin sheaths of nerve fibers [4]. Interaction between LPC and membranes took place in two phases: in the first stage adsorption of LPC on myelin and structural reorganization of the myelin sheaths were observed; in the second stage there was gradual solubilization of the membranes with disturbance of protein—lipid

Mastocytoma P815 Cells (M ± m; n = 6)

% of total

phosphatidylethanolamine		LPC		PC	
experiment	control	experiment	control	experiment	control
24,60±5,24	30,61±4,58	1,78±0,82	0,81±0,67	52,43±2,32*	43,96±2,04
23,01±4,63	30,84±3,75	7,63±1,69*	2,24±1,01	33,76±6,61*	51,32±4,91
28,11±5,50	29,22±1,94	13,27±2,05*	2,97±0,79	16,37±3,45*	41,83±3,91
28,67±3,51	21,87±5,70	15,23±4,58*	2,56±0,80	11,86±7,66*	46,68±9,95

complexes. Taking account of factors of lysophospholipid accumulation during exposure to cytotoxic influences of different kinds (complement [8], endotoxic shock [5]) and pathological processes (inflammation [7], ischemia [11]), it can be tentatively suggested that activation of phospholip ase hydrolysis is one of the nonspecific mechanisms of cell death.

Oxidative action on the cell leads not only to activation of PLA_2, but also to considerable restructuring of the cell membranes, as is shown by a decrease in their relative PC content and an increase in their relative content of sphingomyelin, phosphatidylserine, and phosphatidic acid, starting with 30 min of incubation of P815 with TBHP (Table 1). The functional parameters of membranes, such as permeability and viscosity, depend on the molar ratio of the classes of lipids present in the membrane and, in particular, the cholesterol/phospholipids ratio, the sphingomyelin content, and the degree of saturation of the fatty acids of the phospholipids [13]. It has been shown [9] that the sensitivity of tumor cells to cytotoxic influences is linked with the phospholipid composition of the cell membranes and the negative charge on its surface. An increase in the content of PC and sphingomyelin is accompanied by a decrease in cell resistance.

The increase in PLA_2 activity and the change in the relative content of the various molecular types of phospholipids, which we recorded, may thus be the cause of disturbance of the stability of cell membranes.

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