MODIFICATION OF RADIOSENSITIVITY OF RAT THYMOCYTES BY CHOLESTEROL-ENRICHED AUTOLIPOSOMES

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An essential role in the development of radiation death of lymphoid cells is played by disturbances of the structure and functions of the plasma membrane [8]. One of the most important components of the plasmalemma is cholesterol (Chs). It has been shown that Chs exerts an influence on the structural state of the membrane and the properties of receptors, and it directly or indirectly modulates the activity of many key enzymes. Thus Chs can be regarded as a regulator of the vital activity of the cell. Irradiation is known to lead to changes in Chs concentration in various tissues, and to induce activation of its synthesis in thymocytes and liver cells [5]. It has also been shown that an increase in the content of Chs in membranes of erythrocytes and liposomes prevents the formation of products of lipid peroxidation (LPO), induced by irradiation [6]. It can be postulated on the basis of these findings that enriching lymphocytes with Chs will affect the radiosensitivity of these cells.

The aim of this investigation was to study the possibility of modifying the radiosensitivity of thymocytes as a result of their enrichment with Chs by means of liposomes. To make the transfer of Chs into cells more efficient, we used Chs-containing autoliposomes, prepared from a lipid extract of the thymus. One manifestation of interphase death of lymphocytes caused by exposure to radiation is the internucleosomal degradation of chromatin, accompanied by the formation of low-molecular-weight fractions of polydeoxyribonucleotides (PDN). Another characteristic feature of interphase death of lymphocytes is the development of processes of apoptosis, culminating in disintegration of the cells and pinching off of apoptotic bodies from them [9]. As a result, PDN formation and changes in size of the cells have been used as criteria of radiation damage to thymocytes.

EXPERIMENTAL METHOD

Thymocytes were isolated from the thymus of noninbred albino rats weighing 120-130 g by the method in [3]. Thymocytes in a concentration of $3 \cdot 10^6$ cells/ml were incubated in medium 199 at 37°C. PDN were isolated from the cells by the method in [4]. Lipid extracts of the thymus was isolated by the method in [12] and Chs was added to it up to a molar ratio of Chs/phospholipids = 2. Unilamellar autoliposomes were prepared from thymus lipids by the method in [10]. The malonic dialdehyde (MDA) concentration was determined by formation of its complex with 2-thiobarbituric acid [2]. The DNA concentration was measured by Barton's method [11] and the phosphorus content in phospholipids by the method in [15]. The Chs concentration was determined by high-performance liquid chromatography. The dimensions of the cells were measured on a "Coulter XYZ" counter. The cells were irradiated with x-rays on the RUM-17 apparatus.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that irradiation of the thymocytes in a dose of 10 Gy led to a marked increase in PDN formation, reflecting more intensive degradation of chromatin. Enrichment of the thymocytes with Chs prevents chromatin degradation and PDN formation. With a decrease in the amount of Chs added to the cells in the composition of the auto-

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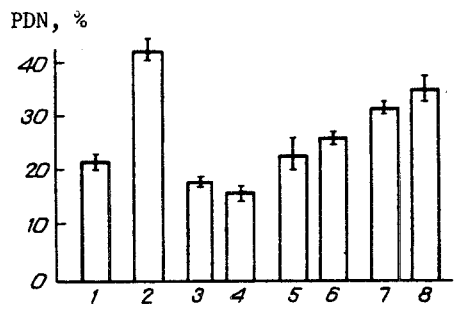

Fig. 1

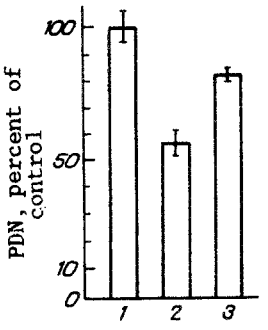

Fig. 2

Fig. 3

Fig. 1. Effect of incubation of thymocytes with cholesterol-containing autoliposomes on radiosensitivity of these cells. Ordinate, yield of PDN, percent of total DNA in cells. Incubation time 4.5 h. Irradiation 30 min after addition of autoliposomes. 1) Control; 2) thymocytes irradiated in a dose of 10 Gy; 3) thymocytes + autoliposomes (500 μg Chs/ml); 4) thymocytes + autoliposomes (500 μg Chs/ml) + irradiation, 10 Gy; 5) thymocytes + autoliposomes (100 μg Chs/ml); 6) thymocytes + autoliposomes (100 μg Chs/ml) + irradiation, 10 Gy; 7) thymocytes + autoliposomes (10 μg Chs/ml); 8) thymocytes + autoliposomes (10 μg/ml) + irradiation, 10 Gy.

Fig. 2. Effect of incubation of thymocytes with previously irradiated cholesterol-containing autoliposomes on PDN formation. Ordinate, yield of PDN, percent of control (control = 100%). Incubation time 4 h. 1) Control; 2) thymocytes + autoliposomes (500 μg Chs/ml), irradiated beforehand in a dose of 10 Gy; 3) thymocytes + autoliposomes (500 μg Chs/ml).

Fig. 3. Effect of enrichment with cholesterol and irradiation of thymocytes on their size. Abscissa, diameter of cells (in μ). Ordinate, number of cells in each fraction (in conventional units). Incubation time 4.5 h. Irradiation 30 min after addition of autoliposomes. Mean value of three experiments shown. 1) Histogram of distribution of dimensions of thymocytes, incubated with autoliposomes (100 μg Chs/ml); 2) histogram of distribution of thymocytes irradiated in a dose of 10 Gy by size; 3) histogram of distribution of thymocytes, incubated with autoliposomes (100 μg Chs/ml), and irradiated in a dose of 10 Gy, by size.

liposomes, its effect on chromatin disintegration caused by irradiation of the cells also was reduced. The radioprotective action of Chs-containing autoliposomes was manifested only if enrichment of the cells with Chs was carried out before irradiation. Addition of the Chs-containing autoliposomes 30 min after irradiation of the thymocytes had no effect on chromatin degradation. In some experiments the quantity of autoliposomes equivalent in phospholipid content, but not enriched with Chs, was added to the cells. In that case the autoliposomes virtually did not reduce the increase in PDN formation induced by irradiation. Hence it follows that the radioprotective effect observed is connected with an increase in the Chs content in the cells. It must be pointed out that during incubation of thymocytes in medium containing autoliposomes in a concentration of 500 μg Chs/ml the yield of PDN was less than in the intact control, whereas in the case of irradiation of Chs-enriched cells this effect was more marked still. During incubation of thymocytes with Chs-containing autoliposomes, irradiated beforehand in a dose of 10 Gy, the yield of PDN also was less than in the control (Fig. 2). LPO products are known to be able to accelerate the passage of Chs into cells [7]. Possibly irradiation, by activating LPO, accelerates enrichment of the cells with Chs, which leads to an increase in their resistance to various unfavorable factors.

Enrichment of the thymocytes with Chs also prevents morphological changes in the cells induced by irradiation. This can be judged from the histogram of dimensions of cells in the population. It was found that during irradiation of thymocytes enriched with Chs, accumulation of apoptotic bodies did not take place (Fig. 3). The diameter of the modified cells increased somewhat, possibly due to the fact that an increase in the Chs content in the plasma membranes of thymocytes leads to an increase in the intracellular Na^+ concentration and to swelling of the cells [13].

TABLE 1. Effect of Enrichment with Cholesterol on Radiation-Induced Malonic Dialdehyde Formation in Thymocytes (M ± m)

Experimental conditions	Concentration of autoliposomes, μg Chs/ml	MDA content in thymocytes, nmoles/mg protein
Control (without incubation)	0	0,216±0,250
Control (incubation)	0	0,750±0,061
+ autoliposomes	100	0,839±0,072*
+ autoliposomes	500	0,901±0,087*
Irradiation, 10 Gy	0	1,441±0,124
Autoliposomes + irradiation, 10 Gy	100	1,019±0,063**
Autoliposomes + irradiation, 10 Gy	500	0,770±0,066**

Legend. Incubation time 4.5 h. Irradiation 30 min after addition of autoliposomes to cells. *$p < 0.05$ compared with unirradiated control, **$p < 0.05$ compared with irradiated control.

Exposure of a cell to radiation is known to activate LPO [1]. Chs itself is not an antioxidant. However, if a component of the membrane, it increases the rigidity of packing of the fatty acid chains of the phospholipids, and this impairs oxygen diffusion and inhibits LPO development [14]. Determination of the content of LPO products forming complexes with thiobarbituric acid, in modified cells, showed that enrichment of thymocytes with Chs prevents the development of LPO induced by irradiation (Table 1). Addition of Chs to thymocytes in the composition of autoliposomes, in a concentration of 500 μg/ml, completely suppressed MDA formation. It is interesting to note that if the concentration of autoliposomes was 100 μg Chs/ml, preventing postradiation degradation of chromatin in the thymocytes, an increase in the MDA concentration was observed.

Thus enrichment of thymocytes with Chs leads to suppression of radiation-induced LPO in them and prevents their damage by LPO products. At the same time, processes of enzymic degradation of chromatin and apoptosis are inhibited.

It is shown for the first time that an increase in the Chs content of thymocytes makes them more resistant to the action of ionizing radiation. This opens up prospects for the use of Chs-containing liposomes as an effective agent for modifying the radiosensitivity of cells.

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