CHANGES IN PROPERTIES OF PLASMA MEMBRANES OF NORMAL AND TUMOR CELLS ASSOCIATED WITH CHOLESTEROL INCORPORATION

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In the modern view, changes in the physical properties of the plasma membrane of cells play an important role in their division processes [5]. An increase in microviscosity of the plasma membranes of fibroblasts and myocytes due to incorporation of cholesterol (Ch) has been shown to stimulate their division [9, 13, 14]. On the basis of these investigations it has been suggested that Ch plays the leading role in the onset of atherosclerotic changes in the early stages of plaque development. Investigations of freely growing cells have shown that tumor cell membranes contain little Ch and differ from normal cells in the increased liquidity of their plasma membranes [7]. The few publications on this subject, indicating correlation between the microviscosity of the plasma membranes and transplantability of tumor cells, are contradictory in nature [3, 8, 11].

The aim of the present investigation was to study correlation between the Ch content in the plasma membrane of normal (lymphocytes) and tumor cells (leukemia L1210 and an ovarian tumor), on the one hand, and activity of enzymes incorporated into the plasma membrane, on the other hand. The effect of incorporation of Ch into and its release from the plasma membranes of tumor cells on their transplantability also was studied.

EXPERIMENTAL METHOD

Noninbred rats weighing 180-200 g and DBA-2 mice weighing 18-20 g, kept on a standard diet, were used. T lymphocytes were isolated from the thymus of male rats [10]. To obtain ascites tumor cells of the ovaries, 10^8 cells were injected intraperitoneally into each of a series of female rats, and the animals were decapitated on the 9th day. An ascites tumor was obtained from the peritoneal cavity and rinsed free from ascites fluid with calcium-free Hanks' solution. To obtain L1210 lymphoblastic leukemia cells, male DBA-2 mice were given an injection of 10^6 cells each, intraperitoneally. The animals were killed on the 5th day and the cells were removed. The cells were counted in a Goryaev chamber. The viability of the cells was determined with the intravital dye trypan blue. Monolayer Ch-lecithin liposomes (Ch-LL) and lecithin liposomes (LL) were obtained from lipid suspensions by sonication in a disintegrator of UZDN-4 type (USSR) [11]. Incorporation of Ch into membranes of tumor and normal cells was carried out by incubating the cells with Ch-LL by a modified method [15]. To extract Ch from the membranes of all the cells tested, they were incubated with LL. The cell suspensions were washed twice to remove liposomes with calcium-free Hanks' solution on a "Ianetzki" K-23 centrifuge at 200g for 5-10 min.

Ch [6] and phospholipids [2] were determined quantitatively in extracts prepared with Folch's mixture [6]. Protein was measured by Lowry's colorimetric method. Activity of Na, K-ATPase and 5'-nucleotidase was measured as the quantity of inorganic phosphate formed during the reaction [12]. The microviscosity of the phospholipid bilayer of normal and tumor cells was estimated from excimerization of the fluorescent probe, pyrene [1]. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

On incubation of the tumor and normal cells with Ch-LL, Ch was incorporated into the submembranes, and during incubation with LL it was removed. In control experiments these cells were incubated with calcium-free Hanks' solution. The optimal time for incorporation of Ch

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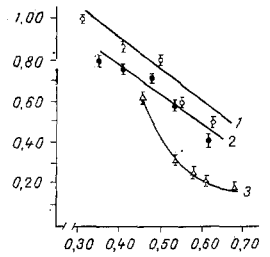

Fig. 1

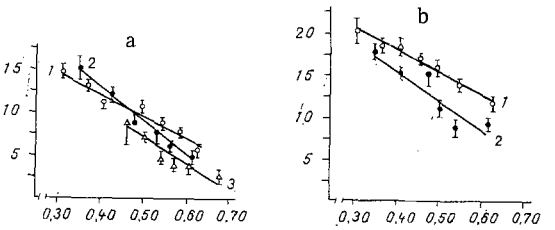

Fig. 2

Fig. 1. Decrease in degree of excimerization of fluorescent pyrene probe accompanied by increase in Ch/PL ratio in L1210 lymphoblastic leukemia cells (1), in ovarian tumor cells (2), and in lymphocytes (3). Abscissa, ratio Ch/PL (moles/mole); ordinate, ratio of intensity of pyrene fluorescence at wavelength 470 nm (maximum of the excimers peak) to intensity of fluorescence at wavelength 392 nm (maximum of monomers peak).

Fig. 2. Changes in Na,K-ATPase activity (a) and 5'-nucleotidase activity (b) during incorporation of Ch into membranes of test cells. Abscissa, ratio Ch/PL; ordinate, enzyme activity (nmoles/mg/min). 1) For L1210 leukemia cells; 2) for ovarian tumor cells; 3) for normal rat lymphocytes.

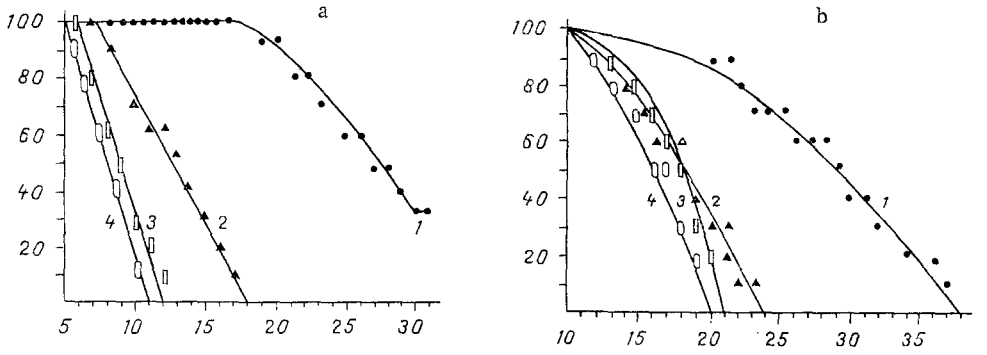

Fig. 3. Period of survival of animals after transplantation of L1210 leukemia cells (a) and ovarian ascites tumor cells (b) differing in their Ch content. Abscissa, time (days); ordinate, number of surviving animals (%). 1) After incubation of cells with Ch-LL; 2) after incubation of cells with LL; 3) after incubation of cells with buffer solution; 4) intact tumor cells.

into plasma membranes was 4 h, and for its removal 2.5-3 h. During incorporation of Ch into the membrane of lymphocytes, ovarian tumor cells, and L1210 leukemia cells, an increase in the Ch content expressed both per milligram protein and per cell was observed. The molar ratio Ch/phospholipids (the Ch/PL ratio) increased under these circumstances for lymphocytes from 0.46 ± 0.02 to 0.68 ± 0.01 (by 53%, $P < 0.001$), for ovarian tumor cells from 0.35 ± 0.01 to 0.62 ± 0.01 (by 77%, $P < 0.001$), and for L1210 leukemia cells from 0.35 ± 0.01 to 0.63 ± 0.01 (by 104%, $P < 0.001$). Ch was removed from native normal and tumor cells, and also from cells into whose membranes Ch was incorporated beforehand. The results of these experiments show that the effectiveness both of incorporation of Ch and of its removal depends on the Ch/PL ratio in the cells: the lower the initial values, the more Ch was incorporated. Removal of Ch, on the other hand, was most effective in the case of cells whose Ch/PL ratio was high. During removal of Ch from Ch-rich membranes of all the cells studied the Ch/PL ratio fell by 40-50%. During incorporation of Ch into and its removal from the cell membranes, no statistically significant change was found in the phospholipid content, expressed either per milligram protein or per cell. Hence it can be concluded that incorporation of Ch and its removal take place as a result of its exchange between liposomes and the cell membrane.

A considerable increase in microviscosity of the phospholipid bilayer of the membranes was discovered in lymphocytes, ovarian tumor cells, and L1210 leukemia cells after incorporation of Ch, as reflected in the degree of excimerization of the pyrene fluorescent probe (Fig.

1). On removal of Ch, an increase in the degree of excimerization of pyrene was observed, evidence of a decrease in microviscosity of the membrane lipids of these cells [1]. An increase in the microviscosity of membranes of normal and tumor cells on account of incorporation of Ch led to a change in activity of the membrane enzymes (Fig. 2a, b). Activity of Na,K-ATPase in the lymphocytes was reduced by 63% ($P < 0.001$), in ovarian tumor cells by 67% ($P < 0.001$), and by 61% in L1210 leukemia cells, whereas 5'-nucleotidase activity was reduced in the ovarian tumor cells by 48% ($P < 0.001$) and in L1210 cells by 40% ($P < 0.001$). After removal of Ch from native normal and tumor cells, no significant changes were found in Na,K-ATPase and 5'-nucleotidase activity. The microviscosity of the cell membranes also was unchanged in this case. On removal of Ch from the Ch-rich membranes of these cells, a significant increase was found in activity of Na,K-ATPase and 5'-nucleotidase up to levels of activity of these enzymes in native cells, accompanied by a decrease in the microviscosity of the lipid layer of the membranes.

It was interesting to study how incorporation of Ch into tumor cell membranes, leading to changes in the properties of their plasma membranes, is reflected in the transplantability of tumor cells. Several series of experiments were carried out for this purpose, in which tumor cells with high Ch/PL ratio (incubated with Ch-LL), cells with low Ch/PL ratio (incubated with LL), control cells (incubated with buffer solution), and intact tumor cells were injected into animals. The length of survival of the DBA-2 mice after transplantation of L1210 leukemia cells after incorporation of Ch into their membranes was increased threefold (Fig. 3a), whereas the length of survival of rats into which ovarian tumor cells rich in Ch were transplanted was doubled (Fig. 3b) compared with the control. When the number of tumor cells in the volume of ascites fluid was counted in the period of tumor growth, a reduction of 2.5-3 times was found in the number of tumor cells in animals of the experimental groups compared with the controls.

The increase in the relative Ch content in the cell membrane thus leads to an increase in microviscosity of the membranes and to a decrease in activity of the membrane-bound enzymes. A decrease in the Ch content, on the other hand, is accompanied by an increase in liquidity of the cell membranes and an increase in activity of enzymes incorporated in them. Incorporation of Ch into plasma membranes of L1210 leukemia cells and ovarian tumor cells sharply reduces their transplantability.

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