ONCOLOGY

Kinetics of Lipid Unsaturation in Blood Plasma of Mice with Lewis Carcinoma

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The extreme nature of changes of lipid unsaturation in plasma of mice with Lewis carcinoma is demonstrated. The maximal activation of lipid peroxidation is observed on days 7-9 after transplantation and correlates with the exponential growth of the tumor. From the 9th day the level of double bonds in plasma lipids increases, this coinciding with the first appearance of metastases in the lungs.

Key Words: *lipid peroxidation; lipid unsaturation; blood; transplanted tumor*

One of the most important mechanisms of homeostasis and nonspecific resistance of the organism is lipid peroxidation (LPO). Alterations in the activity of LPO are the result of various pathophysiological processes and, in turn, lead to restoration of the damage occurring during these processes [1,4]. LPO has been shown to play a role in the regulation of cell proliferation and in the development of pathological processes caused by radiation and xenobiotics, in bacterial and viral infections, stress, carcinogenesis, and atherogenesis, and for the functioning of phagocytes and macrophages. Changes in the system of regulation of LPO occurring during the formation of malignant tumors have been determined [2-4]. The degree of lipid unsaturation of different organs and tissues, including blood lipids, can serve as an integral index of LPO activity in the organism. Previously we found that in patients with malignant tumors the degree of lipid unsaturation in blood plasma and erythrocytes, which is characterized by the number of double bonds in lipids, is much higher than in the control [5,6]. It was demonstrated that the level of double bonds in oncological patients has its own prognostic significance and it was speculated that this is one of the features which characterize the interaction between tumor and organism.

In order to verify this speculation experimentally, we studied the kinetics of double bonds in blood lipids of mice with transplanted Lewis carcinoma.

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MATERIALS AND METHODS

One hundred thirty-two male C57Bl×DBA mice weighing 23-25 g bred at the Stolbovaya nursery were used. Lewis carcinoma was grafted intramuscularly in the hind leg (2×10^5 cells in 0.05 ml of medium 199). Mice were killed by cervical dislocation 1, 3, 6, 24, 72, 168, 216, 288, 336 and 432 h after tumor transplantation. Fifty-two intact mice were used as the control. Two independent experiments were carried out at a 6-month interval, 6-8 mice being used at each experimental point. Blood from intact mice and from those with tumors was taken directly after sacrifice in test tubes with heparin and centrifuged at 2500 g for 15 min. Extraction of the plasma lipids was performed after Folch [9]: 5 ml of a mixture of methanol and chloroform were added to 0.25 ml plasma. After 30 min of shaking, 2 ml distilled water were added and the samples were placed in the refrigerator. Quantitation of double bonds was carried out not earlier than 1 h after the end of extraction in an ADS-3 analyzer

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of double bonds [7], for which purpose 100 μl lipid extract were introduced into the reaction vessel. The number of double bonds in the lipids was calculated per volume of plasma, a stilbene solution being used as the standard.

RESULTS

As is seen from Fig. 1, the growth of Lewis carcinoma is accompanied by a drastic change in the degree of unsaturation of blood lipids. At early stages (3 days after grafting of the tumor) the level of double bonds is the same as in intact animals; the decrease in the number of double bonds during the first 3 h is probably caused by the stress reaction of the organism to the procedure.

During the following period, as the tumor grows, a pronounced drop is noted in the number of double bonds in plasma lipids, and a minimal level of double bonds (to 13% *vs.* the control) is observed on days 7-9 after tumor transplantation. The further development of the tumor process is accompanied by an increase in unsaturation of plasma lipids, and by day 14 the level of double bonds attains 85% of the control (Fig. 1).

Days 7-9 after transplantation is a time of transition of Lewis carcinoma from a localized to a generalized stage. As was shown elsewhere [8], at this time the formation of the first metastatic cells, initiating the growth of metastases in the lungs, is determined in the lungs of mice with grafted Lewis carcinoma. As is seen from Fig. 1, the early dissemination of the tumor process coincides with the reversal of the decrease of the level of double bonds in the lipids; the growth of metastases occurs when the degree of unsaturation of plasma lipids is constantly on the rise.

It can be concluded that such a kinetics of the degree of lipid unsaturation is due to the nonuniform nature of the changes of LPO activity in the process of tumor growth, which, in turn, reflects the role of LPO in the organism's reaction to tumor growth. The initial localized growth of any tumor is the result of the activation of the defense mechanisms of the organism which prevent the growth and generalization of the tumor. Among these, an activation of LPO, caused, for example, by intensified generation of active metabolites of oxygen [3,4], could be of no small importance. A possible consequence might be activation of cellular antitumor immunity. The initial dissemination of the tumor process is apparently caused by "overstrain" and "derangement" of the system of antitumor defense, including a decrease in LPO activity in the organism, which lays a favorable groundwork for the formation of a generalized tumor process.

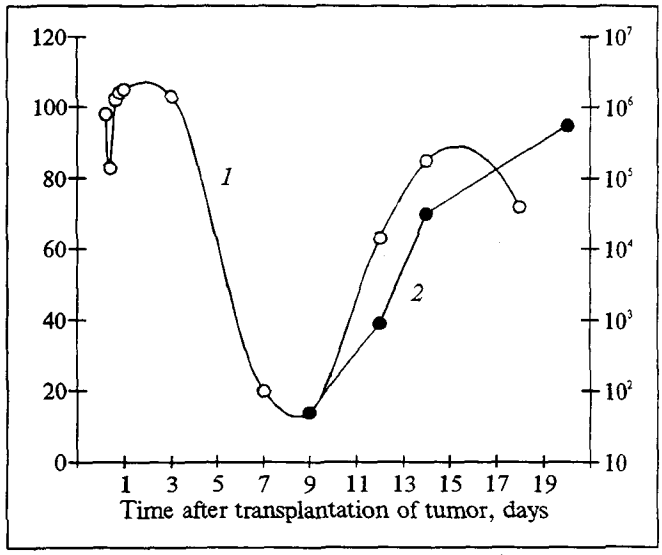

Fig. 1. Changes in the number of double bonds (maen values of 2 experiments) in plasma lipids (*1*) and number of tumor cells in lungs (*2*) in mice with Lewis carcinoma (data from [8]). Ordinate: left – number of double bonds in plasma lipids ($\times 10^{17}$/ml plasma), % control; right – number of tumor cells in lungs.

The findings agree with the results of previous investigations of the degree of unsaturation of blood lipids in patients with breast cancer. It has been shown that in patients with operable (localized) tumors the level of double bonds in blood lipids is reliably lower than in patients with a generalized tumor process [5].

Thus, the results of this study suggest that LPO is one of the mechanisms of antitumor defence of the organism. Evaluation of LPO activity in patients with malignant tumors may be useful for determining the spread of the tumor process and for elaborating the optimal schemes of complex treatment including manipulation of the system of LPO regulation taking into account the stage of tumor growth.

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