GROWTH AND METASTASIZATION OF LEWIS LUNG CARCINOMA IN MICE DURING THE ACTION OF DRUGS ON METABOLISM AND ON EFFECTS OF KININS

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Recent investigations have yielded evidence of differences in the pathogenetic mechanisms of metastasization and primary tumor development [1, 2, 5]. For instance, metastasization of malignant tumors is accompanied by disturbances of vascular permeability, vascular tone, the rheologic properties of the blood, and other changes in the microcirculation, linked with changes in the activity of various regulatory systems of the body and, in particular, the kallikrein-kinin system (KKS) [5]. According to our data [4], the appearance of macroscopically distinguishable metastases is preceded by activation of kininogenesis and of general proteolysis of the blood.

To study the role of the individual components of the KKS (kinins, kinin-forming and kinin-destroying enzymes) in growth and metastasization of Lewis lung carcinoma (LLC), experiments were carried out with the use of parmidine (pyridinol carbamate), a kinin antagonist [6-9], and also with cellulose sulfate, an activator of kininogenesis [8, 12, 13], and unithiol, a kininase inhibitor [7, 11].

EXPERIMENTAL METHOD

Experiments were carried out on 296 C57BL mice of both sexes weighing 20-30 g. LLC was inoculated intramuscularly in the animal's hind limb in a dose of $2 \cdot 10^5$ tumor cells. A tumor that was easily palpable developed at the site of inoculation by the 9th-12th day, and metastases formed by the 19th-21st days.

Parmidine was dissolved in distilled water on heating to 60-70°C and, after cooling, was administered internally to the animals in a volume of 0.2 ml and in doses of 25 to 200 mg/kg either once or in the form of a therapeutic course, starting from the 6th day after inoculation of the tumor. Cellulose sulfate was dissolved in isotonic NaCl solution and injected intravenously in a volume of 0.2 ml and in a dose of 100 mg/kg, causing an abrupt decline of the plasma kininogen level [8]. Unithiol was dissolved in distilled water and given internally in a volume of 0.2 ml, once (dose 150 mg/kg) or fractionally (30 mg/kg each time). In the case of combined administration of parmidine and cellulose sulfate or unithiol, the latter were given 60 min after parmidine. Animals of the control groups (inoculation of the tumor) received distilled water or isotonic NaCl solution in the corresponding volume, administered in the same way and at the same times as the above preparations.

The mice were decapitated on the 21st day after the beginning of the experiment, the weight of the tumor at the site of inoculation and the mean number and size of the lung metastases were determined, after which the percentage inhibition of tumor growth and, in some experiments, the mean duration of survival of the treated animals compared with the control, were calculated by the usual methods. The weight of the metastases (in mg) was determined by the formula [10], assuming that the metastases were spherical and that their density was 1 g/cm^3.

The experimental results were subjected to statistical analysis with calculation of the level of significance of the differences (p).

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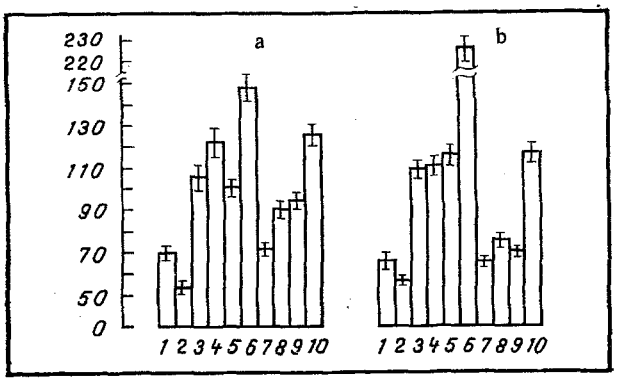

Fig. 1. Changes (in %) in number (a) and weight (b) of lung metastases (in % of control) in mice with LLC under the influence of drugs acting on metabolism and effects of kinins. 1) Parmidine (50 mg/kg, five times); 2) parmidine (100 mg/kg, five times); 3) cellulose sulfate (100 mg/kg); 4) cellulose sulfate (100 mg/kg, twice); 5) unithiol (150 mg/kg); 6) unithiol (30 mg/kg, five times); 7) parmidine (100 mg/kg, five times) + cellulose sulfate (100 mg/kg); 8) parmidine (100 mg/kg, five times) + cellulose sulfate (100 mg/kg, twice); 9) parmidine (100 mg/kg, five times) + unithiol (150 mg/kg); 10) parmidine (100 mg/kg, five times) + unithiol (30 mg/kg, five times).

EXPERIMENTAL RESULTS

Whether given as a single dose or repeatedly, in various doses, parmidine had no significant effect on growth of LLC. Meanwhile, when the drug was given as a therapeutic course (a daily dose of 100 mg/kg) an increase in the mean length of survival of the animals by 25-30% ($p < 0.01$) was observed. These data suggest that parmidine has no direct cytostatic action but it can interfere indirectly in the process of tumor growth.

Administration of cellulose sulfate to the mice in the early stages after inoculation of the tumor (6th and 12th days) inhibited growth of LLC by more than 30%. However, if administered later, this substance did not affect tumor growth. The possibility cannot be ruled out that a definite role in the mechanism of action of cellulose sulfate is played by stimulation of the immune response, which is observed when other sulfate polysaccharides are used [3].

The use of unithiol, an inhibitor of kinin-destroying enzymes (kininases) had virtually no effect on growth of LLC.

Preparations affecting metabolism and the biological effects of kinins had a more marked effect on metastasization of LLC. For instance, the use of parmidine, a kinin antagonist, in a dose of 50 mg/kg daily for 5 days reduced the number of metastases by 31.4% compared with the control ($p < 0.01$). The action of the drug was potentiated when the daily dose was increased to 100 mg/kg (the number of metastases was reduced by 46.3%, $p < 0.01$). The weight of the metastases under these circumstances was reduced by 35.4 and 44.1% respectively (Fig. 1).

By contrast with this, an increase in kinin production or delay in kinin inactivation with the aid of cellulose sulfate and unithiol respectively caused activation of metastasization in the lungs. Cellulose sulfate and unithiol were found to promote the development of metastases in the stage of their formation, i.e., between the 6th and 12th days, but to have no significant effect on the number and weight of the metastatic nodes compared with the control if these drugs were administered on the 15th day after inoculation of the tumor. For instance, cellulose sulfate, if given on the 6th and 12th days, increased the weight of the metastases at the end of the experiment by 12.2% ($p < 0.05$) and the number of metastatic nodes by 22.8% ($p < 0.01$) but it did not affect these parameters when given on the 15th day.

Administration of unithiol in fractional doses (30 mg/kg, five times), beginning on the 6th day after inoculation of the tumor, activated metastasization of LLC in the lungs of the mice sharply. Administration of unithiol on the 15th day, conversely, did not affect the character of metastasization of LLC compared with the control.

These results show that growth and, in particular, metastasization of LLC in mice are associated to a certain degree with a change in activity of the blood KKS. Evidence in support of this view is given by the results of experiments using activation of kinin production by cellulose sulfate and inhibition of inactivation of endogenous kinins by the use of the kininase inhibitor, unithiol. Elevation of the plasma kinin level, accompanying administration of these preparations, evidently creates favorable conditions for transfer of tumor cells into the microcirculatory system of the lungs. Meanwhile parmidine, a kinin antagonist, while not affecting growth of the primary tumor, can inhibit metastasization of LLC, and in all probability can prevent the unfavorable action of high concentrations of kinins on the microcirculation in the lungs.

It is submitted that the treatment of malignant tumors ought to include a combination of pharmacological agents that will take into account the pathogenetic mechanisms of metastasization and factors contributing to the inhibition of this process.

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