ONCOLOGY

Boosting the Antitumor Effect of Doxorubicin by Combined Correction of Hemostasis

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The size of the primary tumor and number of metastases to the lungs in mice with Lewis pulmonary carcinoma treated with doxorubicin were, respectively, 3 and 2.2 times less than in the control. Injection of doxorubicin and heparin led to an 8-fold reduction of the tumor. The number of animals with detected metastases to the lungs and the mean number of metastases per animal were decreased. Supplementation of doxorubicin therapy with a complex of drugs correcting platelet aggregation and the antithrombogenic properties of the vascular wall resulted in a reduction of the number of metastases on the lung surface in comparison with the control and with the animals treated with doxorubicin alone.

Key Words: *vascular platelet hemostasis; doxorubicin; metastases*

The adhesion of tumor cells to blood vessel walls, the formation of an oncogenic-thrombogenic embolus and its entrapment in the microcirculatory bed, followed by the development of metastases depend to a great extent on the antithrombogenic activity of the vascular wall and the characteristics of platelet aggregation [6,9]. The antiaggregation and anticoagulation activity of the wall was previously shown to decline during experimental malignant cell growth, whereas the functional activity of platelets increased [4]. A reduction of the antithrombogenic properties of the vascular wall, namely depressed synthesis and release of prostacycline, antithrombin III, and plasminogen activator into the blood in cancer patients, was revealed in an earlier study [1].

We investigated the relationship between the modification of the function of the hemostasis system and malignant growth of tumor cells during chemotherapy.

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MATERIALS AND METHODS

Experiments were carried out with 275 C57Bl/6 mice aged 6 to 8 weeks. A suspension of Lewis pulmonary carcinoma cells in a concentration of 2×10^6 cells in 0.2 ml normal saline was intramuscularly injected in the femur. The mean survival time was estimated starting from the moment of tumor transplantation till the death of the animal. Metastases on the surface of the lungs were counted visually on days 21-23 of tumor development after the isolated lungs were placed in Bouin's solution. Drug therapy was started 2 days after tumor transplantation according to the following protocol: doxorubicin at 400 μg/kg intramuscularly; heparin at 400 uits/kg intramuscularly; dipyridamole at 0.42 mg/kg orally; phytin at 7 mg/kg orally; glutamic acid at 14 mg/kg orally. Doxorubicin and heparin

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were injected twice a week for 2 weeks, and the other drugs twice daily for 3 weeks. Control animals were administered starch and normal saline in the corresponding amounts. The results were statistically processed using Student's t test and methods of variational statistics.

RESULTS

Dipyridamole, a potent inhibitor of platelet aggregation [8], is known to restore the experimentally reduced antithrombogenic activity of the vascular wall or its activity in atherosclerosis patients, particularly if combined with phytin and glutamic acid [5].

Our studies demonstrated (Fig. 1) that administration of dipyridamole to mice with Lewis pulmonary carcinoma for 3 weeks brought about a twofold, on average, reduction of the number of metastases on the lung surface and a 10-day prolongation of the life of dying animals. Administration of dipyridamole simultaneously with phytin and glutamic acid led to an approximately 2.5-fold reduction of the number of metastses. The results suggest that correction of the hemostasis system may boost the effects of antitumor drugs, while simultaneously mitigating their side effects. For example, the potent antitumor antibiotic doxorubicin activates thrombin production in a tumor-bearing organism and thus aggravates the clot-threatening status of the blood [2,7]. To verify this hypothesis, we added drugs modifying the function of the hemostasis system to doxorubicin therapy of tumor-bearing animals (Table 1).

One hundred percent of the control animals developed tumors with metastases to the lungs after implantation of a tumor cell suspension. In animals treated with doxorubicin the size of the primary tumor was one-third, on average, of that in the controls; metastases on the lung surface were revealed in 85% of animals, the number of metastases being reduced 2.2-fold. In animals treated with doxorubicin and heparin the tumors

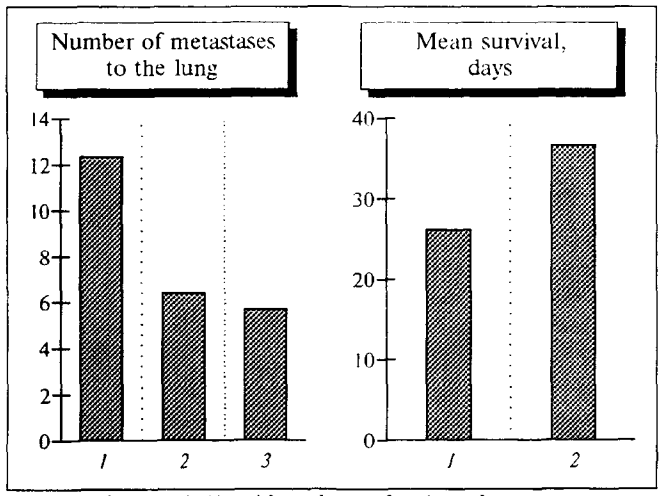

Fig. 1. Effect of dipyridamole on Lewis pulmonary sarcoma metastases in mice. *1*) control; *2*) dipyridamole; *3*) dipyridamole + phytin + glutamic acid.

were 8 times smaller than in the controls. The same percentage of animals developed tumors as in the groups treated with doxorubicin alone, but the fraction of animals with metastases in the lungs was, respectively, 47 and 38% lower as against the controls and animals treated with doxorubicin alone. The addition of a complex of drugs: heparin, dipyridamole, phytin, and glutamic acid to doxorubicin therapy was attended by a further reduction of malignant growth characteristics. The mean size of the tumor did not change, but the number of animals with mature tumors, metastases, and apparent metastases fell 1.6, 1.8, and 2.9 times, respectively, in comparison with the same parameters in animals treated with doxorubicin and heparin.

The findings indicate that administration of heparin in parallel with doxorubicin to tumor-bearing animals appreciably boosts the effect of the antibiotic on both primary tumor growth and the metastasizing process, this being in line with previous reports [3] demonstrating a much higher activity of the doxorubicin-heparin complex in comparison with its separate components. The boosting effect of chemotherapy was, however, the most

TABLE 1. Parameters of Malignant Cell Growth in Tumor–Bearing Animals Treated with Doxorubicin and Drugs Influencing the Hemostasis System ($M \pm m$, $n = 20$)

Experimental conditions	Tumor size, cm^3	Tumor development, %	Number of metastases	Percentage of animals with metastases
Control	17.5±1.1	100±4.3	18.0±1.9	100±4.3
Doxorubicin	5.5±1.1^{+}	87±7.0	8.3±1.8^{+}	85±7.4
Doxorubicin + heparin	2.2±1.0^{+*}	88±6.7	5.6±2.0^{+}	53±10.4^{+*}
Doxorubicin + heparin + dipyridamole + phytin + glutamic acid	2.2±1.1^{+*}	56±10.4$^{+*\circ}$	1.9±1.1^{+*}	30±9.6$^{+*\circ}$

Note. A plus sign shows a reliable difference from the control, an asterisk from doxorubicin alone, and a circle from treatment with doxorubicin and heparin ($p<0.05$).

pronounced according to all the parameters tested when a combination of drugs correcting platelet aggregation activity and the antithrombogenic properties of the cell wall was used.

Hence, the addition of combined correction of the functioning of the hemostasis system to the treatment protocols is advisable for the prevention of metastases; such therapy, including inhibitors of platelet functional activity, adhesion, and aggregation, will improve the anticoagulation activity of the blood and the antithrombogenic activity of blood vessel walls.

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