EFFECT OF IRRADIATION AND CORTISONE ON SPREAD OF METASTASES OF EHRLICH'S CARCINOMA

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A combination of irradiation and hormones, especially corticoid hormones, is widely used for the treatment of tumors. However, these methods of treatment are by no means without their effect on the state of reactivity of the patient. Irradiation may cause acceleration of tumor growth and the more intensive development of metastases [2-4, 7, 12, 13, 15]. Similar results have been obtained in relation to cortusone and its synthetic analogues (1, 5, 6, 8-11, 14, 18, 19].

In the present investigation an attempt was made to use the kinetic characteristics of tumor growth to assess the effect of whole-body x-ray irradiation and of cortisone on the growth and spread of metastases of an Ehrlich's carcinoma.

EXPERIMENTAL METHOD

The investigations were conducted on 165 noninbred mice weighing 18-20 g. Metastasization was produced by injecting 3 million viable tumor cells of an Ehrlich's ascite carcinoma in 0.2 ml of Earl's solution into the caudal vein. The tumor cells were counted in a Goryaev's chamber in diluting fluid containing eosin. The tumor cells which stained with eosin (dead cells) were not counted. Whole-body x-ray irradiation of the mice in a dose of 300 R or a single intramuscular injection of cortisone acetate (Roussel) in a dose of 5 mg was given 48 h before intravenous inoculation of the suspension of tumor cells. The conditions of irradiation were: voltage 180 kV, current 15 mA, filter 0.5 mm Cu + 1.0 mm Al, dose rate 22.5 R/min. Fifteen days after inoculation of the tumor cells the animals were sacrificed and the details of involvement of the internal organs by metastases were recorded. The number of metastases in the lungs and liver (on the surface of the organ and in sections of it 2 mm thick) was counted and their diameter was measured by means of a stereoscopic microscope with an ocular micrometer. The total weight of the metastases in the organ was determined from the formula:

$$M = 0{,}5404 \cdot n \cdot D^3, \tag{1}$$

where M is the total mass of metastases in the organ, n the number of metastases in the organ, and D the mean diameter of the metastases.

In parallel experiments the mean survival period of the experimental animals was determined.

To study the kinetic characteristics of growth of the metastases, Gompertz's equation was chosen as the mathematical model [16, 17]:

$$W_t = W_0 \cdot e^{A/\alpha\,(1-e^{-\alpha t})}, \tag{2}$$

where W_t is the size of the tumor (weight, volume, or number of tumor cells) at the moment of time t; W_0 is the size of the tumor at the point t = 0 (i.e., at the moment of inoculation of the tumor); A and α are constants; e is the base of natural logarithms. According to this equation the doubling time of the tumor is not constant, as during exponential growth, but increases constantly, depending on the size of the tumor

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TABLE 1. Number and size of Metastases and Certain Kinetic Characteristics of Their Growth in the Lungs and Liver of Irradiated Mice and Mice Receiving Cortisone

Group	Metastatic index	Lungs					Liver				
		no. of metastases	mean diameter of metastases (in mm)	W_0 ($\times 10^8$ cells)	γ (in mg/day)	no. of metastases	mean diameter of metastases (in mm)	W_0 ($\times 10^8$ cells)	γ (in mg/day)
Control	2,0	141±15	1,00±0,04	64±4	16,51	—	—	—	—
Irradiation	6,7	203±17	1,15±0,05	113±5	55,05	238±23	1,25±0,05	147±9	102,77
Cortisone	7,6	230±15	1,30±0,03	244±9	56,60	347±14	1,55±0,01	408±13	355,60

Note: The differences are statistically significant ($P > 0.05$).

Fig. 1. Cumulative curves of survival of control (1) and irradiated mice (2) and of mice receiving cortisone (3) with metastases of an Ehrlich's carcinoma. Ordinate — survival rate (in %); abscissa — time (in days).

and the number of times it has doubled. The curve of tumor growth has a horizontal asymptote. The rate of growth of the metastases in the lungs and liver in the animals of the different groups was compared at corresponding (equivalent) points on the kinetic curves of tumor growth.

EXPERIMENTAL RESULTS

The experimental results showed that whole-body irradiation of the animals or administration of cortisone to them caused intensive metastasization of the Ehrlich's tumor. In such animals metastases were found in the lungs, liver, lymph glands, stomach, small intestine, and kidneys. In most cases (40-60%) they were found in the heart and spleen — organs in which metastases of malignant tumors are locallized comparatively rarely. Fluid containing tumor cells frequently accumulated in the peritoneal and pleural cavities. Of all the organs the liver was the worst affected. The metastatic index (ratio between the number of organs with metastases and the number of organs investigated) in the irradiated animals was nearly 3.5 times, and in those receiving cortisone 3.8 times, greater than the corresponding control index (see Table 1). Furthermore, irradiation or administration of cortisone increased the intensity of metastasization in the internal organs. For instance, in the lungs of such animals the metastases were more numerous and larger than in the animals of the control group. In the liver the metastases were usually larger and more numerous than in the lungs. Conditions for growth of metastases in the liver were particularly favorable when cortisone was given (Table 1).

This was evidently the reason for the earlier death of the irradiated mice and of the animals receiving cortisone than of the controls* (Fig. 1). The mean survival period of the irradiated mice (22.0±1.17 days) was only $^2/_3$, and that of the mice receiving cortisone (16.9/ 0.51 days) only $^1/_2$ ($P < 0.01$) the mean life span of the control animals (33.25+1.96 days).

The average kinetic curves of growth of the metastases in the lungs and liver of the irradiated mice and of the animals receiving cortisone are illustrated in Fig. 2. It is easy to see that the experimental kinetic curves (of Gompertz) of growth of metastases in the lungs of these animals diverged from the exponential curves characterizing the growth of metastases in conditions when the magnitude of the factors inhibiting tumor growth is zero to a lesser degree than in the control series. This shows that irradiation and cortisone depress the role of the factors inhibiting growth of the metastases and, thus cureate more favorable conditions for their growth. Comparison of the values of γ obtained by mathematical analysis of the kinetics of growth at the moment when the total weight of the metastases was 0.1 Was† showed the following relationship: irradiation of the animals or administration of cortisone to them before inoculation of tumor cells increased the rate of growth of metastases in the lungs by almost 3.5 times; the metastases in the liver of the mice receiving cortisone grew 3.5 times faster than in the irradiated animals; in both cases the metastases in the liver grew faster than those in the lungs.

*Irradiation or injection of cortisone, carried out on healthy animals, did not lead to their death in the course of the next 3.5 months.

†Was — the value of the total weight of the metastases at the asymptote.

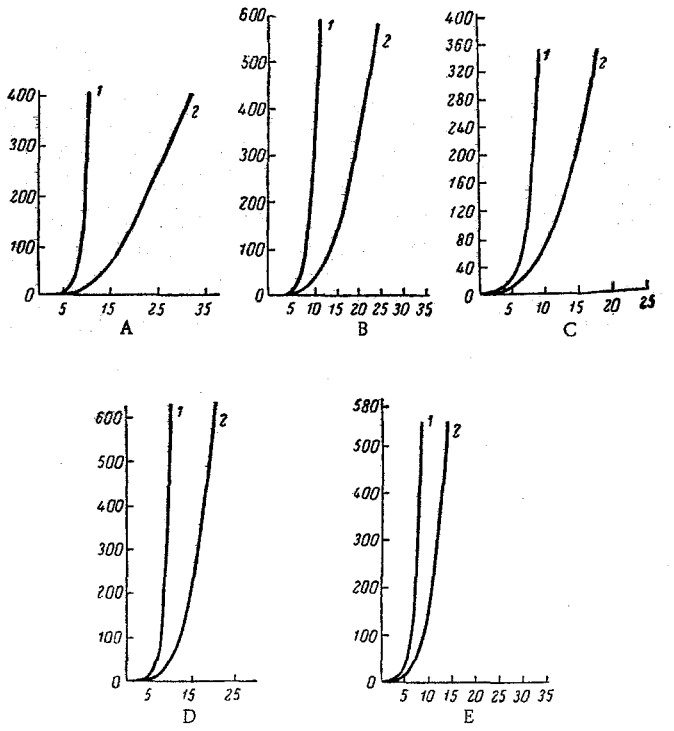

Fig. 2. Averaged kinetic curves of growth of metastases in the lungs and liver of mice. 1) Growth of metastases by an exponential relationship (for comparison); 2) growth of metastases in accordance with Gompertz's function in the lungs of control (A) and irradiated (B) mice and mice receiving cortisone (C) and in the liver of irradiated mice (D) or mice receiving cortisone (E). Absicssa — time (in days); ordinate — weight of tumor (in mg).

Extrapolation of the weight of the metastases on the kinetic curves of the tumor growth to the point t = 0 (i.e., to the moment of intravenous inoculation of the suspension of tumor cells yielded quantitative information concerning the process of adhesion of the tumor cells in the capillaries of the lung and liver.

Irradiation of the animals or administration of cortisone to them greatly increased the adhesion of the tumor cells in the capillaries of the lung. In the irradiated mice, for instance, the number of these cells was nearly twice as high as in the controls. An even greater increase in the number of adherent tumor cells was caused by administration of cortisone. In this case about 250,000 tumor cells were held up in the capillaries of the lung, almost four times as many as the corresponding index (W_0) in the controls (Table 1). It was not possible to judge to what extent irradiation or the administration of cortisone altered the adhesion of the tumor cells in the liver capillaries, for no metastases could be found in this organ on macroscopic or microscopic examination. Comparison of the data for the adhesion of the tumor cells in the liver of the irradiated mice and the mice receiving cortisone shows that administration of cortisone led to the retention of many (2.8 times) more than took place in the irradiated animals.

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