ONCOLOGY

Effects of Chick Embryo Homogenate and Cyclophosphamide on the Growth and Metastatic Spread of Pliss Sarcoma in Rats: A Comparative Study

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A chick embryo homogenate inhibited the growth and metastatic spread of Pliss sarcoma in rats and prolonged their survival. The histological picture of extensive tumor tissue necrosis with a characteristic infiltration by leukocytes and macrophages suggests that the mechanism underlying the antineoplastic action of the embryonal homogenate is quite different from that of cyclophosphamide and involves the activation of cell-mediated immunity.

Key Words: *Pliss sarcoma; embryonal homogenate; cyclophosphamide; cell-mediated immunity*

In the literature, there are reports of the use of embryonal materials for the treatment of breast cancer, pulmonary carcinoma, melanoma, and several other malignant tumors [3,4], but the mechanism of tumor growth suppression by such materials and the nature of their active principle remain unknown. The antitumor activity of embryonal preparations might be linked with the oncofetal proteins they contain, elevated titers of which in adults are usually associated with malignancy [1].

We have compared the effects of a tissue homogenate prepared from 6-day chick embryos and of the chemotherapeutic agent cyclophosphamide on the growth, metastatic spread, and histological picture of Pliss sarcoma in rats.

MATERIALS AND METHODS

A total of 52 Wistar rats of both sexes (body weight 150-200 g) maintained on a standard laboratory diet were used. They were each inoculated subcutaneously into the side with 0.1 ml of a conventionally prepared [5] 50% suspension of Pliss sarcoma cells.

The sources of embryonal tissue were 6-day chick embryos from eggs incubated at 37°C in a humid atmosphere. The embryos were washed with glacial Hanks' balanced salt solution and homogenized in a glass homogenizer in the cold.

Four of the five test groups of rats were treated with the chick embryo homogenate (CEH) using different regimens, while one group was treated with cyclophosphamide (CP); the control group with Pliss sarcoma did not receive any treatment (Table 1). The surviving rats were decapitated under ether anesthesia 25 days after tumor inoculation. The internal organs of all rats were thoroughly inspected to detect metastases. The tumor that had developed at the inoculation site was excised and its linear dimensions and weight were measured. Tumor tissue specimens were subjected to histological examination.

The criteria used to evaluate the treatment results were degree of tumor growth inhibition, incidence of metastases, and survival rate.

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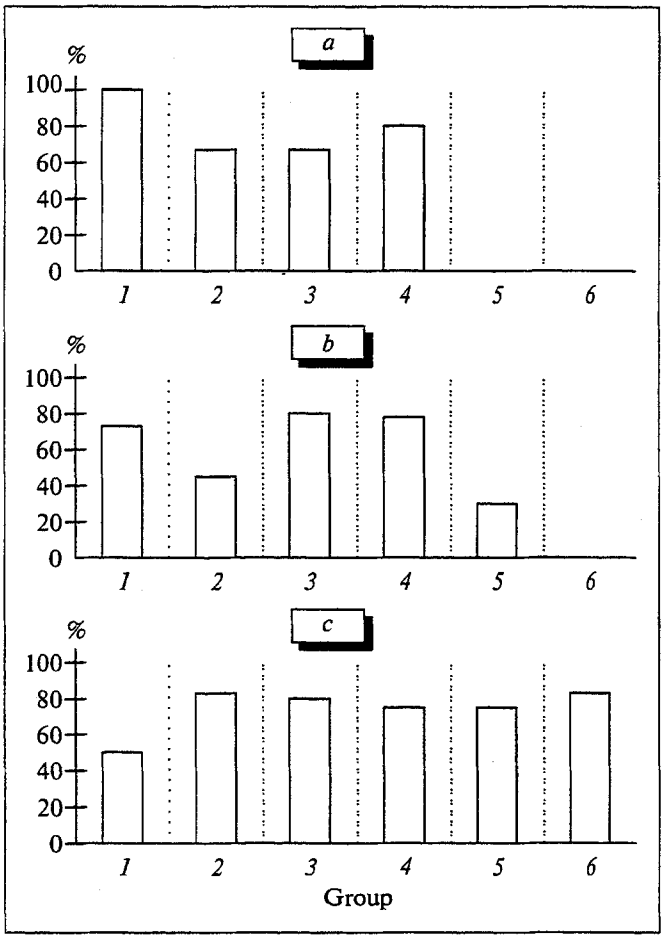

Fig. 1. Impact of different treatment regimens on survival (*a*), tumor growth (*b*), and metastases (*c*) in rats with experimental Pliss sarcoma. Experimental conditions for the different groups of rats are indicated in the Table 1.

The degree of tumor growth inhibition was calculated in percent by the formula: $[(M_C - M_M)/M_C]\times100$, where M_M is the mean tumor mass in a given test group and M_C is the mean tumor mass in the control group.

The incidence of metastases was calculated in percent by the formula: $(R_{Met}/R_{Tot})\times100$, where R_{Met} is the number of rats found to have metastases and R_{Tot} is the total number of rats in a given group.

The survival rate was also determined in percent using the formula: $(R_{Sur}/R_{Tot})\times100$, where R_{Sur} is the number of rats that had survived to day 25 after tumor inoculation and R_{Tot} is the total number of rats in a given group.

Histological examination was performed by the standard procedure [2].

RESULTS

The survival rate in all four groups treated with CEH was high, whereas none of the control or CP-treated rats survived to day 25 postinoculation (Fig. 1, *a*). CEH exerted a well-defined inhibitory effect on tumor growth (Fig. 1, *b*). The best results were obtained with daily injection of the embryonal preparation, starting on day 5 or day 10 after tumor inoculation (these two regimens led to 80% and 78% inhibition of tumor growth, respectively). In the group given only one dose of the homogenate 24 h before tumor inoculation, tumor growth was inhibited nearly twice as much (by 73%) as in the group given it once in the same dose at the time of tumor inoculation (45% inhibition), the difference being statistically significant ($p<0.05$). In the CP-treated group, tumor growth inhibition was only 30%, which is significantly below the inhibition level in any of the CEH-treated groups ($p<0.05$).

Neither CEH nor CP had an appreciable effect on the metastatic spread of an already formed tumor (Fig. 1, *c*), although the incidence of metastases in group 1 treated with CEH was only 50% compared to 83% in the control group ($p<0.05$).

Tissue specimens for histological examination were obtained from the tumor inoculation site in all 52 rats. In the control group a picture typical of round-cell sarcomatous tumors was observed. In some cases, a few small areas of necrosis surrounded

TABLE 1. Treatment Regimens Used in the Six Groups of Wistar Rats with Experimental Pliss Sarcoma

Group	*n*	Preparation	Dose	Injection site and route	When injected and how often
1	8	CEH	0.4 ml	Side, S.C.	24 h before tumor inoculation, once
2	6	CEH	0.4 ml	Side, S.C.	Concomitantly with tumor inoculation, once
3	6	CEH	0.4 ml	Tumor	On day 5 after tumor inoculation and then daily
4	10	CEH	0.4 ml	Tumor	On day 10 after tumor inoculation and then daily
5	10	CP	100 mg/kg	I.P.	On day 2 after tumor inoculation and then every other day
6	12	-	-	-	Controls

Note. CEH = chick embryo homogenate; CP = cyclophosphamide.

by small numbers of macrophages, leukocytes, and plasma cells were present, with densely packed tumor cells lying at the periphery of these areas.

A different picture was presented by the specimens from CEH-treated rats. Extensive necrotic foci containing tumor cells with pronounced degenerative changes were seen in all specimens, surrounded and threaded in almost all of them by richly vascularized connective tissue structures. There was total or focal necrosis with infiltration by macrophages, lymphocytes, neutrophils, plasma cells, mast cells, fibroblasts, and fibrocytes.

In the CP-treated group, even rats with a clinically recorded tumor growth inhibition had developed a sarcomatous tumor that tended to invade the fatty and muscular tissues. Necrotic foci were not apparent in any of the specimens examined. All rats in this group were found to have intestinal necrosis at autopsy.

The results of this study demonstrate an antineoplastic action of CEH by a mechanism distinct from that of CP. As documented histologically, this embryonal preparation, unlike CP, not only inhibits tumor growth but also causes extensive tumor necrosis. Moreover, there was no evidence of toxicity in the form of intestinal necrosis such as was observed in all the CP-treated animals. The presence of lymphocytes and macrophages in necrotic areas may, in our view, be regarded as an indication that the embryonal homogenate predominantly stimulates cellular mechanisms of antitumor resistance.

REFERENCES

1. G. I. Abelev, *Ontogenez*, **20**, № 6, 607-615 (1989).
2. R. D. Lillie, *Histopathologic Technique and Practical Histochemistry*, McGraw-Hill, New York (1965).
3. R. L. Ol'shanetskaya, *On the Treatment of Malignant Tumors with Placental and Embryonal Tissue Extracts* [in Russian], Kharkov (1939).
4. L. V. Polezhaev, *Loss and Recovery of Regenerative Capacity by Organs and Tissues in Animals* [in Russian], Moscow (1968).
5. Z. P. Sof'ina *et al.* (Eds.), *Experimental Evaluation of Antineoplastic Agents in the USSR and USA* [in Russian], Moscow (1980).

The Metastatic Potential of Tumors Depends on the pH of Host Tissues

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pH-Metric characteristics of Ehrlich's ascitic carcinoma and of a protein-induced tumor were compared in mice at different stages of the neoplastic process from tumor cell inoculation to the animal's death. On the acidographic curves, the time of change from acid to alkaline pH values coincided with that at which the tumors began to metastasize. It is suggested that this finding may be of use for the early diagnosis of tumor metastases.

Key Words: *metastases; pH; tumor strain; acidosis; alkalosis; mice*

Metastases from tumors are a top-priority clinical problem in oncology and are the subject of a voluminous literature. However, in none of the studies published so far has the metastatic process in tumor bearers been specifically considered in relation to the pH values of their tissues, although it would seem quite logical to look for the cause of metastases in the active response of these tissues.

The interest of researchers in the pH of tumor tissue is reflected in the literature only by data on single pH measurements in various tumors [2-4], and the authors of some publications have made an at-

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