EFFECT OF CYCLOPHOSPHAMIDE ON BLOOD SERUM α-FETOPROTEIN LEVEL IN MICE WITH HEPATOMA 22a AT DIFFERENT TIMES OF TUMOR DEVELOPMENT

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KEY WORDS: α-fetoprotein; hepatoma; cyclophosphamide.

It is stated in the literature that the effect of antitumor preparations depends on the period of tumor development [2, 3, 5, 6]. However, it is not yet clear whether these are universal principles characteristic of all tumors and, in particular, of transplantable hepatoma 22a.

One sign of development of hepatoma in man and animals is α-fetoprotein (AFP) production. It was accordingly decided to study the effect of cyclophosphamide (CP) on the serum AFP concentration in mice with hepatoma 22a, depending on the stage of development of the tumor.

EXPERIMENTAL METHOD

Experiments were carried out on 800 male C3HA mice weighing 22-25 g, into which a hepatoma 22a was transplanted after adaptation for 14 days to illumination from 8 a.m. to 8 p.m. and a regular feeding rhythm (food was given to the animals ad lib at 4 p.m.). The strain of hepatoma 22a was obtained from the Laboratory of Experimental and Tumor Strains, Oncologic Scientific Center, Academy of Medical Sciences of the USSR.*

A suspension of tumor cells in physiological saline (1:3) was injected subcutaneously into the mice in the lateral part of the trunk. To obtain an adequate number of animals with hepatoma, the tumor was reinoculated 3 times at intervals of 9 days.

There were two series of experiments. In series I the effect of CP on AFP production by hepatoma cells was studied when the cytostatic was given on the 6th, 7th, 11th, and 16th days of tumor development (1st-4th groups respectively), and group 5 served as the control. CP was injected subcutaneously in a single dose of 150 mg/kg body weight at 11 a.m. The AFP level was determined in the control and experimental animals daily at 11 a.m.-12 noon during 3 or 4 days of the experiment. In series II CP was injected subcutaneously into the animals of group 1, once only on the 6th day of tumor development (150 mg/kg body weight), into the animals of group 2 on the 4th day (150 mg/kg), and into those of group 3 it was injected twice, on the 4th and 6th days of tumor development (100 mg/kg body weight each time) at 12 noon-2 p.m. The mice of group 4 served as the control. The AFP content was studied daily from the 6th through the 13th days of growth of the hepatoma at 11 a.m.

The AFP content in the blood serum was determined by double immunodiffusion in agar using a standard test system by Ouchterlony's method in Khramkova and Abelev's modification [1]. Fetal mouse blood serum in a dilution of 1:120 was used as the test antigen and monospecific antiserum against mouse AFP in a dilution of 1:2 as the test antiserum. The sensitivity of the test system was 0.5 mg%. Monospecific antiserum against mouse AFP was obtained by immunizing rabbits with pooled mouse fetal serum followed by absorption with adult mouse serum. The specificity of the resulting antiserum was verified by comparison with antiserum against mouse AFP obtained from the Laboratory of Immunodiagnosis of Cancer, Oncologic Scien-

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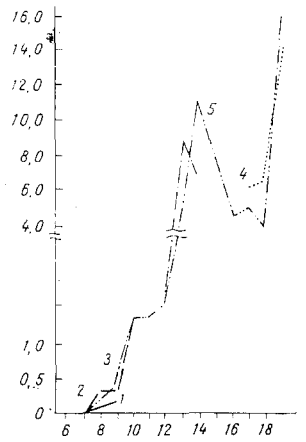

Fig. 1

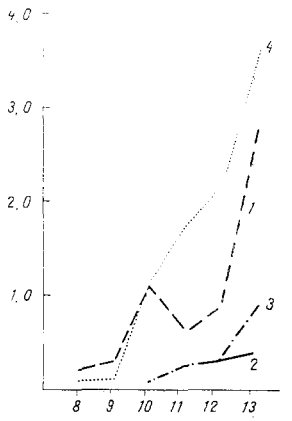

Fig. 2

Fig. 1. Serum AFP concentration in mice with hepatoma 22a depending on time of injection of CP. Abscissa, days of hepatoma development; ordinate, AFP concentration (in mg%). 1) CP injected on 6th day; 2) on 7th day; 3) on 11th day; 4) on 16th day; 5) control.

Fig. 2. Effect of CP on AFP level in blood serum of mice with hepatoma 22a depending on time of development of tumor: 1) CP injected on 6th day, 2) on 4th day, 3) on 4th and 6th days; 4) control. Remainder of legend as to Fig. 1.

tific center, Academy of Medical Sciences of the USSR.* The results were subjected to statistical analysis by the Fisher-Student method, at the $P \leqslant 0.05$ level of significance.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that AFP appeared in the blood of the control mice in series I on the 8th day of tumor development. Later its level rose progressively up to a maximum on the 14th day. It then fell (to the 18th day), but on the 19th day of tumor growth it rose sharply. These results agree with those obtained previously [4]. The action of CP, injected into the animals in a dose of 150 mg/kg, differed at different stages of tumor development (series I; Fig. 1). If CP was injected on the 6th day of growth of the hepatoma, AFP appeared in the blood on the 8th day of its development, but its level on the 9th day was only one-quarter as high as in the control ($P < 0.004$). Injection of CP on the 7th day of tumor growth did not change the AFP concentration on the following days compared with the control. Injection of CP on the 11th day of development of the hepatoma caused an increase in the AFP concentration after 2 days to twice the control level ($P < 0.02$), but after 3 days its level was 34% lower ($P = 0.05$). Injection of CP on the 16th day of tumor growth also led to an increase in the AFP level by 67% after 2 days ($P = 0.05$).

In the control animals of series II AFP was found in the blood serum on the 8th day of hepatoma development. The protein concentration increased gradually until the 13th day (Fig. 2). If CP was injected on the 6th day of tumor growth AFP also was found on the 8th day of the growth cycle of the hepatoma. At that time and on the 9th-11th day of tumor development, no significant differences were found in the AFP concentration in the blood compared with the control. On the 12th day of growth of the hepatoma in animals receiving CP, however, the AFP level was 59% lower ($P = 0.037$) than the control. At the next stage of the experiment the AFP concentration did not differ significantly from the control. When CP was injected on the 4th day of tumor development, AFP was found for the first time only on the 12th day after inoculation of the hepatoma. At this and the next stage of the experiment (13th day) its level after injection of CP was reduced to 13.7 and 11.1% respectively of the control value ($P < 0.02$ and < 0.001; Fig. 2). If CP was injected twice, namely on the 4th and 6th days of tumor growth, the appearance of AFP in the blood was observed on the

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10th day of hepatoma development. At all times of the investigation its level in the experimental animals was significantly lower than in the control ($P < 0.001$), especially after 12 days of growth of the tumor (Fig. 2).

The results are evidence that, first, CP influences the time of appearance of AFP and its blood level in animals with hepatoma 22a and, second, that the character of the changes in AFP concentration depends on the stage of tumor development in which the cytostatic was injected. On the 4th day of the growth cycle of the hepatoma CP had the most effective action on AFP; this suggests that it is at this stage of tumor development that the population of AFP-producing cells arises. CP injected into animals at this stage of tumor development delays the formation of the AFP-synthesizing cell population.

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INHIBITORY ACTION OF A COMBINATION OF NEURAMINIDASE AND INTERFERON IN MICE WITH RAUSCHER LEUKEMIA

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KEY WORDS: Rauscher leukemia; neuraminidase; interferon.

There are many reports in the literature of the inhibitory effect of the enzyme neuraminidase on growth of various types of neoplasms [2, 5, 9]. The effectiveness of action of the enzyme is due to its ability to enhance the specific immune response of the host organism to tumor-bound antigens. The successful use of interferon and, in particular, highly purified and concentrated preparations of interferon, in experimental and clinical oncology has been the subject of recent reports [3, 4].

In 1976 the present writers published the results of a study of the effect of neuraminidase from *Vibrio cholerae* on development of Rauscher leukemia in mice [1]. In particular, it was shown that preliminary treatment of virus-containing spleen cells from affected mice with this enzyme had an inhibitory action on the onset of the disaese following intraperitoneal injection of these cells into healthy animals.

The object of the present investigation was to study the combined action of neuraminidase and interferon on the development of Rauscher leukemia.

EXPERIMENTAL METHOD

BALB/c mice weighing 16-20 g were used. Rauscher's leukemia virus was first passed through mice of this line by intraperitoneal inoculation of 0.1 ml of a suspension of spleen

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