IMMUNOTHERAPEUTIC USE OF NEURAMINIDASE IN CHEMICALLY INDUCED CARCINOGENESIS

I. N. Maiskii, G. P. Airapet'yan, G. D. Kobrinskii, N. A. Troitskaya, G. B. Gudkova, T. A. Agafonova, and Academician V. D. Solov'ev*

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The action of neuraminidase from Vibrio cholerae on the development of sarcomas induced in CBA mice by dimethylbenzanthracene was studied. Administration of the enzyme preparation began after the appearance of tumors in the animals. Administration of 50 units neuraminidase twice a week to the mice for 3 months was effective in the early stages of carcinogenesis. An increase in the survival period of the experimental mice compared with the control animals was observed after intraperitoneal injection of cells of a syngeneic induced sarcoma, and of injection of sensitized lymphocytes obtained from syngeneic mice with tumors into the region of the developing tumor. No positive effect was observed if lymphocytes injected into the region of the tumor were treated previously with neuraminidase. Simultaneous injection of neuraminidase into the region of the developing tumor and intraperitoneal injection of syngeneic induced sarcoma cells treated with the enzyme was particularly effective against carcinogenesis. The possibilities of the use of the enzyme neuraminidase under clinical conditions are discussed.

KEY WORDS: chemical carcinogenesis; neuraminidase; immunotherapy

A new and promising direction in the immunotherapy of malignant disease is the use of the enzyme neuraminidase in experimental oncology.

There have now been many reports of the comparatively high effectiveness of neuraminidase as a factor increasing immunogenicity and reducing the oncogenicity of several transplantable tumors [4, 5, 7].

The present writers first discovered in 1974 that direct injection of the enzyme into the tumor in an autologous system has the effect of inhibiting the development of chemically induced sarcomas [1].

The inhibitory action of the enzyme neuraminidase was studied in the investigation described below in relation to the conditions of its administration in chemically induced carcinogenesis.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice into which 0.4 mg of the carcinogen dimethylbenzanthracene in 0.2 ml of peach oil was injected into the thigh muscle. Immunotherapy began 8-10 weeks later, when induration appeared at the site of injection.

The source of the neuraminidase was a filtrate of a culture of Vibrio cholerae containing 500 units of enzyme per milliliter. Neuraminidase activity was determined by Warren's method [8]. The animals were treated with the enzyme by different methods. In the series of experiments in which neuraminidase was injected twice a week directly into the region of the palpable induration, the sessional dose of the enzyme was 50 units per mouse.

*Academy of Medical Sciences of the USSR.

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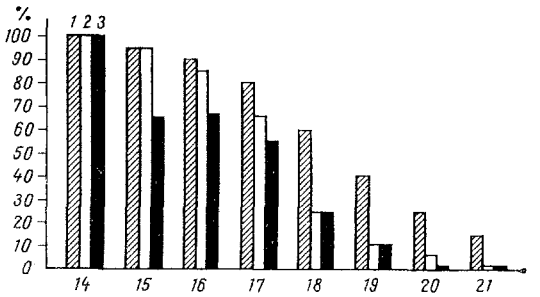

Fig. 1. Results of neuraminidase treatment at different stages of carcinogenesis. Here and in Figs. 2 and 3: abscissa, stage of development of induced tumors (in weeks); ordinate, % of surviving animals. 1) Early stage; 2) stage of formed tumors; 3) control.

When tumor cells previously treated with neuraminidase were used, they were incubated in the proportion of 50 units of enzyme to 1×10^6 sarcoma cells for 15 min at 37°C. The tumor cells incubated with the enzyme were injected intraperitoneally three times, each in a dose of 5×10^5 viable cells at intervals of 1 week.

In the series of experiments in which lymphocytes were used for combined treatment, they were obtained from syngeneic animals with tumors in the early stages of carcinogenesis. These cells were isolated from the spleens of the mice and purified in a Ficoll-Verografin density gradient of 1.077 [2]. The resulting suspension was divided into parts and one part was incubated with neuraminidase as described above. An injection of 5×10^5 cells treated with neuraminidase or native lymphocytes was then given into the region of the developing tumors.

Mice treated by the same method with neuraminidase inactivated by heating to 56°C served as the control for all the experimental groups.

The Kolmogorov–Smirnov criterion was used for statistical analysis of the results and values were considered to be significant if $\lambda^2 \geq 1.84$ [3].

EXPERIMENTAL RESULTS

The dependence of the inhibitory action of neuraminidase on the stage of development of the tumor was studied in the experiments of series I.

In this series 150 CBA mice were divided into three groups, with 50 mice in each group, 8 weeks after injection of the carcinogen. Group 1 consisted of mice in which a small area of induration could be felt at the site of injection of the carcinogen; group 2 consisted of animals with a visible tumor 0.5-0.8 cm in diameter; group 3 served as the control. Twice a week the animals of groups 1 and 2 were given injections of neuraminidase in a sessional dose of 50 units per mouse into the region of the limb where the nodule could be palpated. The mice of the control group were treated with enzyme inactivated by heat. The duration of the course was 3 months.

The results in Fig. 1 show that dissimilar effects were observed after injections of neuraminidase into the region of the developing induced tumors. If treatment of the animals began in the early stages of tumor development the percentage of surviving animals was significantly higher than in the group of mice in which neuraminidase injections were given at the stage of formed tumors, and higher still compared with the control.

In view of data in the literature indicating an increase in the immunogenicity of the cells of transplantable tumors as a result of treatment with neuraminidase, analogous experiments were carried out with tumors induced by a carcinogen.

To obtain a greater immunotherapeutic effect, and allowing for the killer function of sensitized lymphocytes, in the experiments of series II 80 CBA mice were given combined treatment.

The results of these experiments are given in Fig. 2. Group 1 consisted of animals which received intraperitoneal injections of cells of a syngeneic induced sarcoma, previously incubated with neuraminidase, starting

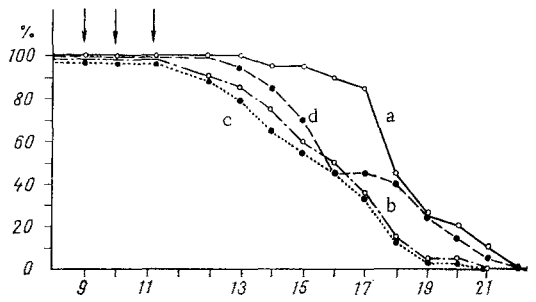

Fig. 2

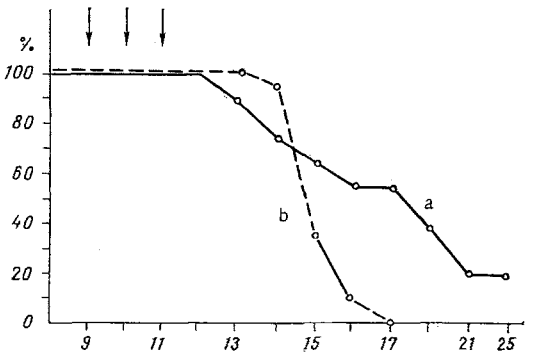

Fig. 3

Fig. 2. Effectiveness of combined treatment on growth of induced sarcomas: a) injection of tumor cells treated with neuraminidase and of syngeneic native lymphocytes; b) injection of tumor cells and lymphocytes treated with neuraminidase; c) injection of lymphocytes treated with enzyme; d) control. Injection of cells indicated by arrows.

Fig. 3. Effectiveness of single injection of neuraminidase into growing sarcoma and of tumor cells treated with the enzyme (injected intraperitoneally): a) experiment; b) control. Injection of neuraminidase and of cells indicated by arrows.

from the 9th week of carcinogenesis, and sensitized lymphocytes obtained from syngeneic mice with tumors were injected into the region of the developing tumors.

As Fig. 2 shows, the effect of treatment in experimental group 1 was observed during the 6 weeks after the last treatment, for in 85% of the animals the survival period exceeded the control by 4 weeks, which is statistically significant.

In these experiments an attempt also was undertaken to treat not only the cells of induced sarcomas, but also sensitized lymphocytes, with neuraminidase. The basis for this procedure was the hypothesis that in this way it is possible to abolish the blocking components which might prevent the killer function of the lymphocytes of the animals with tumors. These animals formed group 2, in which the experiment began in the same way as in group 1, except that the lymphocytes were first incubated with neuraminidase. Sensitized lymphocytes treated with neuraminidase were injected into the animals of group 3.

As Fig. 2 shows, treatment of the animals with tumors in this way gave a weaker effect than in the control group. These results are explained by the observations of Woodruff et al. [9], who showed that removal of sialic acids from erythrocytes, platelets, and lymphocytes by neuraminidase shortens their life span, disturbs their biological functions, and causes their rapid removal from the blood stream. The action of neuraminidase was thus effective only against tumor cells.

With these findings in mind, the last series of experiments (III) was carried out in which chemically induced carcinogenesis in CBA mice was treated by two methods simultaneously: injection of neuraminidase directly into the tumor nodules and intraperitoneal inoculation of syngeneic sarcoma cells previously incubated with neuraminidase.

It will be clear from Fig. 3 that this triple treatment had a considerable inhibitory action on carcinogenesis. By the time of death of all the control animals, which was observed in the 17th week, 55% of the mice in the experimental group still survived. After the next 8 weeks of carcinogenesis 25% of the animals were still alive (period of observation).

In conclusion a number of important factors influencing the effectiveness of neuraminidase treatment must be mentioned. When neuraminidase is injected directly into the region treated with the carcinogen, its action must be prolonged, for after its end the malignant cells may once again form tumors. The stage of carcinogenesis is important for the immunotherapeutic effect of the enzyme: In the early stages the effect of neuraminidase is more favorable. Incubation of the cells of a tumor induced by a carcinogen with neuraminidase leads to an increase in their immunogenicity, just as was observed in experiments with primary tumors.

The mechanism of action of the enzyme has not yet been fully explained and the generally accepted views were described in an earlier paper [1].

Meanwhile, it must be pointed out that, according to the evidence of Rosato et al. [6], intradermal injection of malignant cells of varied histogenesis, previously treated with neuraminidase, into 25 cancer patients gave an immunotherapeutic effect.

The results now described, together with those of other investigators, suggest that neuraminidase can be used as an immunotherapeutic agent not only experimentally, but also clinically.

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EFFECT OF PRECURSORS OF ENDOGENOUSLY SYNTHESIZED CARCINOGEN DIMETHYLNITROSAMINE ON ACTIVITY OF ITS DEMETHYLASE IN RAT LIVER

E. D. Karpilovskaya and B. L. Rubenchik

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Peroral administration of dimethylamine, sodium nitrite, and a combination of these two precursors of endogenously synthesized dimethylnitrosamine to rats increases the activity of the demethylase of this carcinogen in the liver microsomes. Under chronic experimental conditions the addition of dimethylamine to the rats' diet stimulates demethylase activity even if the diet contains casein, an inducer of this enzyme system. Actinomycin D, an inhibitor of protein synthesis, prevents the increase in demethylase activity in the microsomal fraction induced by dimethylamine.

KEY WORDS: demethylase; dimethylamine; dimethylnitrosamine; sodium nitrite; endogenous synthesis; rat liver microsomes

Much attention has recently been paid to the possibility of formation of the carcinogenic agent dimethylnitrosamine (DMNA) in vivo from sodium nitrite ($NaNO_2$) and amines (or amides) [5, 6]. However, no attempt has so far been made to consider whether these precursors of endogenously synthesized DMNA have any effect on microsomal demethylase, with which the mutagenic and carcinogenic action of this compound is linked [3, 7]. The study of the factors controlling demethylase activity and synthesis in the microsomes would help to elucidate the mechanism of action of DMNA, one of the most active carcinogens and one which causes tumors in six species of laboratory animals.

The object of this investigation was to study the effect of dimethylamine (DMA), $NaNO_2$, and a combination of these two precursors of endogenously synthesized DMNA on the activity of the demethylase of this nitrosamine in the rat liver.

Laboratory of Dietary Carcinogenic Factors, Kiev Research Institute of Dietary Hygiene, Ministry of Health of the Ukrainian SSR.

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