INHIBITORY ACTION OF NEURAMINIDASE OF *Vibrio cholerae* IN RAUSCHER MOUSE LEUKEMIA

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The inhibitory action of the neuraminidase of *Vibrio cholerae* in Rauscher mouse leukemia was studied. After treatment of the spleen cells of leukemic mice with neuraminidase in doses of 50 units/ml or more, the ability of these cells to induce leukemia when injected into BALB/c mice was inhibited significantly. Neraminidase in the above concentration, if given by repeated parenteral injection, had no therapeutic action in Rauscher leukemia.

KEY WORDS: *Neuraminidase of* Vibrio cholerae*; Rauscher mouse leukemia.*

Work on the study of the inhibitory action of neuraminidase of *Vibrio cholerae* on growth of some experimental malignant tumors has recently been published [3, 4, 6].

The discovery of regression of fibrosarcoma in mice following injection of cells of these tumors treated with neuraminidase *in vitro* is also pertinent to this issue [4, 5]. Solov'ev et al. [1] studied the action of this enzyme on chemically induced carcinogenesis and showed that injection of neuraminidase prevents the appearance of sarcomas inducible experimentally in mice by the action of the carcinogen DMBA. The action of neuraminidase has also been tested on experimental passage of L 1210 leukemia in mice [2]. After treatment of leukemic cells with a neuraminidase preparation and subsequent injection of these cells into DBA mice the oncogenicity of the leukemic cells was sharply reduced although they retained their immunogenicity, as shown by the resistance of the mice to subsequent infection with intact L 1210 cells.

The etiological role of oncornaviruses has been established reliably in certain leukemias of animals and, in particular, in several leukemias of mice.

In this investigation the inhibitory action of neuraminidase of *V. cholerae* was studied in one such leukemia — Rauscher leukemia.

EXPERIMENTAL METHOD

BALB/c mice weighing 16-20 g were used. Rauscher mouse leukemia virus was passed through a series of BALB/c mice by intraperitoneal inoculation of 0.5 ml of a 10% suspension of spleen cells from mice with Rauscher's leukemia from a previous passage. The development of leukemia was deduced from splenomegaly which appeared in the infected mice on the 17th-19th day after inoculation. Pathomorphological investigations on a sample of spleens from leukemic animals also were carried out. The final analysis of the results of inoculation was made on the 40th day of infection. In three experiments to study the inhibitory action of neuraminidase different batches of preparations of Soviet *V. cholerae* neuraminidase obtained by L. K. Shataeva and G. D. Kobrinskii at the Leningrad Institute of Macromolecular Compounds, Academy of Sciences of the USSR, were used. The activity of the preparation varied in the different series from 100 to 500 units/ml. In the fourth experiment a prepara-

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TABLE 1. Inhibitory Action of Preparations of *V. cholerae* Neuraminidase on Onset of Rauscher Leukemia in Mice

Expt. No.	Dose of neuraminidase, units/ml	Number of mice		P
		total	with leukemia	
1	10	50 (expt.) 150 (control)	46 120	>0.05
2	20	40 (expt.) 20 (control)	33 20	>0.05
3	50	80 (expt.) 20 (control)	36 19	<0.01
4	50	35 (expt.) 20 (control)	9 15	<0.01

Legend. Neuraminidase preparation of USSR origin used in experiments Nos. 1, 2, and 3, American preparation in experiment No. 4.

TABLE 2. Use of *V. cholerae* Neuraminidase to Treat Mice Infected Experimentally with Rauscher Leukemia

Expt. No.	Activity of neuraminidase preparation injected, units/0.1 ml	Number of mice		P
		total	with leukemia	
1	50	30 (expt.) 20 (control)	27 20	>0.05
2	50	30 (expt.) 20 (control)	27 15	>0.05

Legend. Preparation of Soviet origin used in experiment No. 1, American preparation in experiment No. 2.

tion of *V. cholerae* neuraminidase from Calbiochem (USA), with an activity of 500 units/ml, was used for comparison.

The neuraminidase preparations were mixed with a suspension of spleen cells from mice with Rauscher leukemia (concentration 2.5×10^7 cells/ml). The concentration of the preparation in the suspension varied in the different series of experiments from 10 to 50 units/ml. After incubation of the infected cells with the neuraminidase preparations at 37°C for 45 min the mixture was injected into BALB/c mice in a dose of 0.5 ml. In control experiments cells of leukemic mice were incubated in a similar way with physiological saline and also injected intraperitoneally into mice. As an additional control of toxicity of the neuraminidase preparations in each series of experiments preparations of this enzyme were injected intraperitoneally into animals in the concentration given above. The significance of differences between the development of leukemia in the mice of the experimental and control groups in each series of experiments was determined by the χ^2 criterion.

In two other experiments to study the treatment of mice infected with Rauscher leukemia, preparations of neuraminidase in a concentration of 50 units/ml were injected intraperitoneally in a dose of 0.1 ml on the first, third, fifth, seventh, and ninth days after infection of the animals. The results were compared with those for the development of Rauscher leukemia in control animals which did not receive this "treatment."

EXPERIMENTAL RESULTS

The results of the experiments to study the inhibitory action of the various *V. cholerae* neuraminidase preparations in mice with Rauscher leukemia are given in Table 1.

As Table 1 shows, the neuraminidase preparations in a dose of 50 units/ml had a statistically significant inhibitory action on the onset of Rauscher leukemia in BALB/c mice. Both the preparations of Soviet origin and that obtained from Calbiochem (USA) proved to be

effective. In doses of 10 and 20 units/ml, however, the enzyme preparations had no such action. It is important to note that in all the experiments the *V. cholerae* neuraminidase preparations in concentrations of 10-50 units/ml were nontoxic for BALB/c mice when injected intraperitoneally. In view of these findings it was possible to study the therapeutic action of neuraminidase preparations in Rauscher's leukemia, and the results are summarized in Table 2. They indicate that no therapeutic effect of neuraminidase could be obtained even by the use of massive doses of the preparation.

These investigations thus demonstrated the marked inhibitory action of *V. cholerae* neuraminidase preparations in Rauscher mouse leukemia. In these experiments, just as in investigations by other workers [2], only massive doses of the enzyme (50 units/ml) gave any effect. A dose of 20 units/ml in the present experiments was too low, unlike in experiments with transplantable L 1210 leukemia [2].

The *V. cholerae* neuraminidase used for the treatment of infected mice was ineffective even if concentrated preparations of the enzyme were injected repeatedly; this evidently indicates the importance of direct contact between concentrated preparations of neuraminidase and tumor cells. Treatment of tumor cells with the enzyme leads to liberation of sialic acids [2] and also of certain substances of low molecular weight, bound with the cell glycoproteins. This process, by reducing the negative charge on the surface of the leukemic cells, facilitates contact between them and the immunocompetent cells of the host [6, 7].

These investigations using Rauscher mouse leukemia as a model, together with studies of the inhibitory action of *V. cholerae* neuraminidase preparations in chemical carcinogenesis and experimentally transmitted L 1210 leukemia by other workers, point to the possible use of this enzyme for the investigation of problems connected with the immunotherapeutic approach to the treatment of leukemia in man.

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