INHIBITORY ACTION OF VIRUSES OF THE TICK-BORNE ENCEPHALITIS COMPLEX DIFFERING IN NEUROVIRULENCE ON MOUSE TUMORS IN VIVO

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The study of the oncolytic properties of viruses has shown [8] that, of 42 viruses tested, only 11 influence the growth of one or more tumors. A special place is occupied by viruses of the tick-borne encephalitis (TBE) complex: Russian spring-summer encephalitis (the eastern variant is implied) and louping ill. These viruses have been tested on many tumors and have yielded a higher percentage of positive results than other viruses [1, 3, 4, 6, 7].

Considering that in previous investigations strains of viruses possessing a high degree of neurovirulence were used in every case, in the present series the object chosen was to study the inhibitory action of strains of different degrees of neurovirulence on tumors. The connection between the inhibiory action of viruses and the period and mode of their adminstration of the animal was also studied.

EXPERIMENTAL METHOD

The viruses used in the investigation included strains of TBE viruses highly pathogenic toward albino mice (Sofyin, Krok, Khabarovsk-17), the Nurch strain of the western variant of TBE virus, strain K-va of virus of Omsk hemorrhagic fever, strain TP-21 of Malayan Langat virus, and its attenuated variant—TP-21/326 [2]. All the strains were used in lethal doses in the form of a virus-containing suspension of mouse brains.

Mice aged 1.5-2 months and weighing 16-18 g were used for transplantation of the tumors. Ehrlich's ascites carcinoma and Crocker's sarcoma were the test tumors selected. The first was used in the ascites and solid forms and the tumor material was injected subcutaneously. Usually 0.2 ml of a cell suspension obtained from mice weighing 16-18 g on the 7th day after being inoculated with the tumor was injected. This volume of suspension contained 1-3 million viable cells. The cells of the Crocker's sarcoma were obtained by trypsinization of a tumor nodule taken from a mouse on the 7th-10th after inoculation. In this case 0.5 ml of suspension containing 500.000 tumor cells was injected. This dose of cells guaranteed successful inoculation of the tumor in 100% of cases [5].

EXPERIMENTAL RESULTS

After passage of the viruses in the tumors, as Table 1 shows, the greatest fall in transplantability of the ascites carcinoma was caused by strains Sofying and Nurch of TBE virus and by strain K-va of Omsk hemorrhagic fever virus. In the case of interaction with cells of Crocker's sarcoma, the strains of TBE virus (Sofyin, Krok, Nurch) likewise were most active, with strain K-va exhibiting a lower degree of activity. Strain TP-21 of Malayan Langat virus had only a weak action on the change in transplantability of the tumor cells both of the ascites carcinoma and of the sarcoma.

To study the inhibitory properties of the strains Sofyin, TP-21, and the variant of the latter TP-21/326 against Crocker's sarcoma and Ehrlich's ascites carcinoma, virus-containing material in the form of a barin suspension was injected into the tumor in a dose of 100.000 LD_{50} (Table 2).

Table 2 shows that the inhibitory action of the tested strains was exhibited against both tumors. Growth of Crocker's sarcoma was inhibited equally by all three strains used. After injection into a subcutaneously inoculated Ehrlich's carcinoma strain Sofyin had the greatest effect on growth of the tumor

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TABLE 1. Transplantability of Mouse Tumors in Vivo after a Single Passage of Virus through Them

Tumor	Transplantability (in %)					
	original	after passage of strain				
		Sofyin	Krok	Nurch	K-va	TP-21
Ascites carcinoma inoculated intraperitoneally	100	0	50	0	0	73
Ascites carcinoma inoculated subcutaneously	100	0	50	0	23	77
Crocker's sarcoma	100	27	27	23	50	70

subcutaneously inoculated Ehrlich's carcinoma strain Sofyin had the greatest effect on growth of the tumor cells. Strains TP-21 and TP-21/326 also inhibited growth of this tumor, but to a lesser degree.

In the case of intraperitoneal injection of the cells, strain Sofyin produced considerable inhibition of growth of the ascites carcinoma (1 ml of ascites fluid compared with 6 ml in the control), strain TP-21 had less effect on growth (3 ml), and the volume of tumor ascites fluid in the animals injected with strain TP-21/326 was just the same as in the control animals.

To study the relationship between the action of the viruses in inhibiting growth of the tumor cells and the age of the infected tumor, strains Sofyin and TP-21 were injected into an Ehrlich's ascites carcinoma inoculated subcutaneously and intrapertioneally, and into a Crocker's sarcoma, at various times ranging from 24 h to 5 days after inoculation of the tumor cells.

The inhibition was the stronger the earlier the virus was injected into the tumor tissue. Injection of strain Sofyin into the Crocker's sarcoma 24 and 48 h after inoculation of the animals with the tumor cells, for instance, inhibited the growth of the tumor by 67% or more compared with the control. Injection of this strain in the same dose into more mature tumors (72, 96, and 120 h) caused less inhibition of their growth, and the tumors of all three ages were equally sensitive to the action of the virus. Similar results were obtained also with strain TP-21.

In relation to the Ehrlich's ascites carcinoma inoculated subcutaneously, a direct relationship also was observed between the degree of inhibition of tumor growth and the time of injection both of strain Sofyin and of strain TP-21. Subcutaneous injection of strain Sofyin into the site of injection of the tumor cells of an ascites carcinoma 24, 48, and 72 h later led to death of the animals before the 16th day, counting from the moment of inoculation of the tumor, with signs of developing encephalitis. Strain TP-21, injected in the same way into an animal with an inoculated tumor in the same dose and at the same time as the strain Sofyin, did not cause death of the animals.

Whatever method was used to inject them into the animal, all 3 strains were found in the tumor tissue. The greatest degree of inhibition of tumor growth of the Ehrlich's carcinoma was observed with strains Sofyin, TP-21, and TP-21/326 when injected intracerebrally into the animals. In relation of Crocker's sarcoma the greatest effect was observed with the Sofyin strain when injected into the tumor, and with strains TP-21 and TP-21/326 when injected intracerebrally. For all three strains peripheral injection of

TABLE 2. Inhibitory Action of Three Strains of Viruses of the Tick-Borne Encephalitis Complex on Growth of Tumors in Mice

Tumor	Strain of virus	Age of tumor (in days)	Interval between inoculation of tumor and injection of virus (in h)	Number of animals	Size of tumor (in mm^2), M±m	
					after injection of virus	in control
Crocker's sarcoma	tumor	16	96	6	942±39,2	1180±37,3
	TP-21	16	96	6	942±39,2	1180±37,3
	TP-21 326	16	96	6	942±39,2	1180±37,3

the virus proved less effective in inhibiting growth of the tumors: subcutaneosuly for the ascites carcinoma and intraperitoneally for the solid tumors.

The study of the metabolic activity of the tumor cells (by the color test) showed that strain Sofyin lowered the metabolic activity of the infected cells by 90%. Strain TP-21 and its variant TP-21/326, in analogous conditions, altered the metabolic activity of the tumor cells to a lesser degree.

The Ehrlich's ascites carcinoma in the control animals consisted of round tumor cells with clear borders, large nuclei, and a narrow band of cytoplasm. The number of cells in active mitosis in the various phases of division was 25-30 per 1000. Propagation of strains Sofyin, TP-21, and TP-21/326 after injection into 4-day old tumor tissue was accompanied by changes in the morphological appearance of the cells.

The Sofyin strain completely arrested the mitotic activity of the cells within 24 h of its injection into the tumor. With strains TP-21 and TP-21/326 some increase in the mitotic activity of the cells was observed 1 day after, and inhibition of mitotic activity 2 days after injection of these strains. At this time binuclear cells began to appear.

TBE and Langat viruses and the attenuated variant of the latter, by interacting with tumor cells, are thus capable of influencing the proliferative activity of these cells. This is shown by the complete loss or diminution of transplantability of the tumors and also by delay in their growth.

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