

CHANGES IN THE ONCOLYTIC PROPERTIES OF THE SERUM OF RATS WITH GUÉRIN'S CARCINOMA UNDERGOING TREATMENT WITH SARCOLYSIN

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The use of the oncolytic reaction for assessing the effectiveness of the various methods of treatment of tumors has been investigated, from reports in the literature, only to a very slight degree. Only from isolated pieces of research has data been obtained on the oncolytic activity of the sera of cancer patients before and after surgical removal of the tumors. There are, thus, indications that after the radical surgical treatment of cancer patients the oncolytic activity of the serum increased. In cases where a nonradical operation was performed the oncolytic activity remained at a low level, and as the clinical course of the disease became aggravated, it fell still further [2, 4-7]. The changes in the oncolytic activity of the sera of animals and man with malignant disease treated by chemotherapy, so far as we can judge from the accessible literature, has not been studied at all.

Nowadays, when chemotherapy is beginning to occupy an important place in the treatment of certain forms of malignant disease, the search for objective methods of evaluation of the efficacy of drugs is a task of some urgency. From observations of an increase in the oncolytic properties of the sera of cancer patients after their successful surgical treatment, it may be thought that the reaction of oncolysis might serve as one such method.

In this paper we describe the results of one of our experiments directed toward the verification of this hypothesis. In the research we used a method of determination of the oncolytic properties of serum which we ourselves suggested, based on the principle of photometry [1].

EXPERIMENTAL METHOD

Fourteen rats with a positive oncological serum index i.e., their sera possessed the property of lysing tumor cells) were inoculated subcutaneously with a suspension of cells of a Guérin's rat carcinoma in a dose of 0.5 ml. On the 7th day after inoculation of the tumors, when they had reached measurable dimensions (mean diameter 6.5 mm*), we began to treat all the rats of this group with sarcolysin in a dose of 5 mg of the drug per kg body weight. Altogether 7 intraperitoneal injections of sarcolysin were given to each animal at 72-hour intervals. In the course of sarcolysin treatment the oncolytic activity of the serum was measured periodically.

* We measured the tumor with calipers in 3 directions: length (along the length of the rat's body), width (across the rat's body) and height of the tumor, in mm; we then calculated its mean diameter from the formula $\frac{3abc}{a+b+c}$, where a, b and c are the length, width and height of the tumor respectively.

TABLE 1

Oncolytic Indices of the Sera of Rats with Guérin's Carcinoma Treated with Sarcylisin, and of Control Rats.

Serial No.	Rat No.	Oncolytic index				
		before tumor inoculation	after inoculation of tumor			
			6th day	9th day	21st day	27th day
Rats with Guérin's carcinoma, treated with sarcylisin						
1	55	+15	-18	16	+14	+4
2	65	+13	-16	-10	-	+9
3	66	+49	-20	-20	+5	+11
4	46	+12	-15	-9	+4	+3
5	48	+7	-2	0	+7	+10
6	64	+7	-7	-3	-1	-4
7	50	+6	-12	-12	+10	+3
8	42	+11	-14	-11	+10	+6
9	45	+35	-16	-9	-8	+4
10	67	+37	-14	-14	+10	+27
11	68	+77	-17	-17	+3	-
12	51	+12	-14	-16	+10	+8
13	71	+28	-16	-14	+11	+13
14	29	+6	-17	-10	+6	+6

Healthy rats receiving sarcylisin

1	37	+11	+10	-16	+12	+2
2	49	+39	+39	-5	+7	+5
3	58	+4	+6	-15	+15	+32
4	47	+6	0	-16	+12	+5
5	33	+15	+5	-14	+9	+2
6	34	+21	+18	-13	+5	+2
7	27	+3	+4	-12	+18	+23
8	26	+30	+31	+31	+19	+20
9	30	+7	+7	-17	+14	+7
10	70	+5	+4	-18	-	-3
11	56	+7	+8	-12	+14	+6
12	69	+6	+10	-17	-	-
13	72	+41	+40	-19	+8	+31

Rats with Guérin's carcinoma, not receiving sarcylisin

1	36	+14	-17	-16	0	0
2	23	+15	0	+1	0	-16
3	25*	+28	+6	+13	0	Died
4	16	+8	-10	-9	-7	-9
5	39	+41	-18	-17	-17	-20
6	13	+36	-15	-12	-10	-11
7	18	+73	-10	-6	-11	-15
8	62*	+11	-16	-17	-11	Died
9	31	+6	-11	-10	-9	-19
10	24*	+7	-9	-10	-7	Died
11	21	+35	+10	+18	+5	-12
12	17	+5	-9	-7	-20	-29
13	22	+8	-20	-8	-8	-15
14	38	+6	-20	-19	-20	-21

* Rats Nos. 25, 62 and 24 died from the tumor.

As controls we took 2 further groups of rats (13 animals in one group and 14 in the other). The oncolytic index of the sera of all the rats included in the two positive. The animals of one of these groups were inoculated subcutaneously with a suspension of cells of a Guérin's rat carcinoma at the same time as the rats of the experimental group and also in a dose of 0.5 ml. The second control group consisted of rats which received no tumor inoculations, but in which the injections of sarcylisin and measurements of the oncolytic activity of the sera were carried out at the same times as in the experimental groups.

EXPERIMENTAL RESULTS

The experimental results obtained are shown in Tables 1 and 2 and the figure.

As can be seen from Table 1, in all the rats of the first group, before inoculation of the tumor, the sera possessed well-marked oncolytic activity (shown by positive figures). On the 6th day after inoculation of the tumor the oncolytic index (OI) was in all cases negative, which indicated a sharp fall in the oncolytic activity of the sera.

On the 9th day after inoculation of the tumor, depression of oncolytic activity was observed as before; the OI in all cases was negative. On the 21st day the OI of the serum of all the animals except two (rats Nos. 45 and 64) once more became positive, which was evidence of an increase in the oncolytic activity of these rats. In 4 rats (Nos. 29, 42, 48 and 55) the oncolytic activity was completely restored to its initial (before inoculation of the tumor) level. In only the two rats (Nos. 45 and 64) did the OI continue to fall. On the 27th day a further increase in the OI of the sera was observed in 5 animals (Nos. 45, 48, 66 and 71); in another 5 rats (Nos. 42, 50, 51, 55 and 65) the OI, on the other hand, fell slightly, and in rats Nos. 29 and 46 it remained at its former level. So far as the control animals were concerned, whose sera also possessed well-marked oncolytic activity, in the group of rats in which the tumor was not exposed to the action of sarcylisin a sharp fall in the oncolytic activity was observed on the 6th day after inoculation of the tumor, and this had not recovered by the time the experiment ended. In the group of animals which were not inoculated with the tumor but which received sarcylisin, on the 6th day the oncolytic activity of the serum was unchanged in the majority of the rats, and only in one (rat No. 47) was it decreased. On the 9th day the OI of the serum in all the rats except one (rat No. 26) fell sharply. On the 21st day the serum of all the animals tested had again acquired the property of lysing tumor cells. On the 27th day, by comparison with the previous days of

TABLE 2

Mean Diameters of Tumors in Rats with Guérin's Carcinoma and Treated with Sarcolysin, and in Control Rats

Serial No.	Rat No.	Mean diameters of tumor at different times after inoculation (during sarcolysin treatment)		
		7th day	14th day	26th day
		in mm		
Rats with Guérin's carcinoma and treated with sarcolysin				
1	55	9,1	11,0	7,4
2	65	9,5	10,2	12,1
3	66	9,4	13,0	7,1
4	46	11,0	13,2	9,7
5	48	10,1	8,4	4,2
6	64	9,9	6,8	3,6
7	50	8,5	7,4	7,2
8	42	4,8	6,8	6,5
9	45	6,8	6,8	4,9
10	68	9,2	18,1	7,3
11	68	4,5	7,5	4,2
12	51	8,9	9,9	7,5
13	71	8,2	10,1	8,7
14	29	8,9	6,5	0

Rats with Guérin's carcinoma and not treated with sarcolysin

1	36	5,0	21,5	30,2
2	23	9,1	23,2	31,0
3	25*	7,8	23,0	Died
4	16	7,3	21,0	34,2
5	39	5,4	20,2	45,0
6	13	4,8	10,0	43,0
7	18	5,3	17,4	39,0
8	62*	7,7	24,4	Died
9	31	9,4	29,3	48,0
10	24*	6,9	20,0	Died
11	21	9,3	24,0	34,0
12	17	7,9	22,4	44,0
13	22	6,5	21,8	45,0
14	38	6,2	21,8	36,2

* Rats Nos. 25, 62 and 24 died from the tumor.

restoration of the oncolytic power of the sera of these animals.

As may be seen from curve II, the administration of sarcolysin to the animal itself caused a fall in the oncolytic properties of the serum; this fall, however, was only temporary. On the 18th-24th day the animals became habituated to sarcolysin and the oncolytic activity of their sera again rose. The fall in the oncolytic activity of the sera under the influence of sarcolysin must be taken into consideration when analyzing the changes

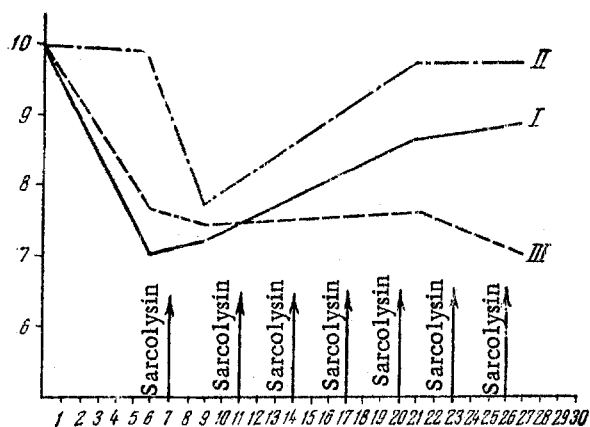
the investigation, some rats showed a slight fall in the OI; in others on the other hand, the value of the OI rose slightly, apart from in rat No. 70 in which the value of the OI at this time was -3. In other words, on the 27th day the serum of all the rats except one (rat No. 70) had once again acquired the property of lysing tumor cells.

The results of the experiment thus showed that in animals with tumors and treated with sarcolysin, on the 21st day an increase was observed in the oncolytic activity of their sera, which persisted until the animals' death (as the result of the development of an epizootic). In the animals with tumors and not treated with sarcolysin a steady loss of the oncolytic properties of the serum was observed. The oncolytic activity of the sera of normal rats under the influence of sarcolysin at first fell. This fall was, however, of short duration, since on the 21st day the sera of all the animals had regained the property of lysing tumor cells.

In Table 2 are shown the results of measurement of the tumors in rats belonging to the first and third groups. We see that on the 26th day the tumor developing in one of the rats treated with sarcolysin (rat No. 29) had completely absorbed. The tumors in the remaining 9 animals, although they had not undergone absorption, had nevertheless begun to regress; in one rat the dimensions of the tumor were unchanged on the 26th day, and only in one (rat No. 65) had the tumor at this time slightly increased in size. In the group of animals with tumors and not treated with sarcolysin, the tumors in all the rats grew rapidly and continuously. As a result of growth of the tumors, 3 rats died on the 26th day.

From a comparison of the results shown in Tables 1 and 2 it will be seen that the increase in the oncolytic power of the sera of the treated animals on the 21st and 27th days was accompanied by a decrease in the size of the tumors on the 27th day. Conversely, the continuous fall in the oncolytic activity of the sera in the group of animals not treated with sarcolysin was accompanied by a steady rise in the dimensions of their tumors. A certain increase in the oncolytic activity of rats Nos. 24, 25 and 62 on the 27th day may evidently be explained by the fact that the oncolytic index of animals is raised before death, as was shown by V. I. Sachkov [3].

For greater clarity, the experimental results are depicted in the form of curves on the figure. Along the ordinate axis are plotted the indices of oncolytic activity of the sera (coefficient of definition 10), and along the abscissa the time elapsing after inoculation of the tumor (in days). Curve I shows a sharp fall on the 6th day. After the 9th day it rises to reach its maximum height on the 27th day. This peak of the curve corresponds to the period when the tumors were reduced in size or were completely absorbed. This clearly suggests that sarcolysin had a definite effect on the animals of this group, inhibiting growth of the tumor and favoring the



Trend of the changes in the oncolytic activity of the sera of rats with Guérin's carcinoma and treated with sarcolysin. I) Changes in the oncolytic activity of the sera of rats with tumors under the influence of sarcolysin; II) changes in the oncolytic activity of the sera of normal rats under the influence of sarcolysin; III) changes in the oncolytic activity of the sera of rats with tumors and not treated with sarcolysin.

in this activity in animals with tumors, treated with sarcolysin. Presumably if sarcolysin did not have the action of lowering the intensity of oncolysis, curve I would have been higher (the oncolytic activity of the corresponding sera would have been higher). Curve III illustrated the continuous fall in the oncolytic activity of the sera of animals with tumors, not treated in any way.

It can thus be concluded that if sarcolysin treatment has an inhibitory action on the growth of the tumor, this is quickly reflected in the state of the oncolytic power of the sera of the treated animals: an increase in their oncolytic activity is observed; conversely, as the malignant disease progresses, the oncolytic properties of the serum rapidly diminish.

In our view the results obtained suggest that the determination of the oncolytic activity of the sera of animals in the course of treatment by a particular antitumor preparation enables the effectiveness of the drug under test to be judged.

SUMMARY

An attempt was made to employ the oncolytic reaction to evaluate the efficacy of sarcolysin treatment in Guérin's rat carcinoma. The oncolytic properties of the sera were periodically investigated in rats suffering from this tumor and treated with sarcolysin. The results obtained were compared with the changes in the oncolytic properties of the serum of animals with tumors untreated with this preparation. It was demonstrated that the rise of the serum oncolytic activity in the treated animals is associated with the reduction of the tumor size. Conversely, the activity fall in the serum oncolytic activity in rats suffering from tumors not treated with sarcolysin is associated with the continuous growth of the tumor. It appears that determination of the oncological properties of the serum during sarcolysin treatment of animals suffering from tumors would enable the efficacy of this therapy on the tumor to be evaluated.

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* Original Russian pagination. See C.B. Translation.