THE MECHANISM OF THE ANTITUMOR ACTION OF DL-N-DI-(2-CHLOROETHYL) AMINOPHENYLALANINE (SARCOLYSIN) AND OF TRIETHYLENIMINO-S-TRIAZINE (TEM)

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The investigation of the mechanism of action of active antitumor preparations of the chloroethylamine and ethylenimine groups on tumor and normally proliferating tissues is an important task in the chemotherapy of tumors.

Preliminary work with one of the first compounds of the chloroethylamine group – methyl-di-(2-chloroethyl)-amine showed that there is a great deal of evidence in support of the direct action of this preparation or of its conversion products on susceptible tissues. For example protection of the bone marrow and the receptor field of the gastrointestinal tract from the action of chlorethazine, circulating in the blood stream, prevents the development of aplasia of the bone marrow and of the vomiting reflex, for in the course of this time the preparation is either combined or inactivated in the tissues of the body [2, 4, 7].

New compounds of the chloroethylamine and ethylenimine groups have now been prepared, whose physicochemical properties and the strength and spectrum of whose antitumor action differ considerably from each other, depending on the "carrier" of the active alkylating groups [2].

Investigations of the blood concentration of the active antitumor preparations sarcolysin and TEM, which we carried out in 1955-1956, showed that it rapidly falls in the first few minutes after administration. We established this in cross-circulation experiments, when after injection of large doses of sarcolysin and TEM to the donor, the recipient rabbit did not die, nor did it show any severe lesion of the hemopoietic system, provided that it received the donor's blood at least 3-5 minutes after administration of the preparation to the donor.

It has been shown [9] that the blood concentration of TEM, labeled with C^{14} in the ethylenimino group, falls sharply, and between 30 seconds and 3 minutes 90-95% of the induced radioactivity disappears; about 1% of the radioactivity stays in the blood for roughly 6 hours. The curve of fall in concentration of radioactivity after administration of labeled TEM, according to Nadkarni and Goldenthal, is identical with that of methyl-di-(2-chloroethyl)-amine (chlorethazine).

Great differences between chlorethazine and novochlorethazine sarcolysin and TEM on the other were revealed in experiments in which the circulation of the bone marrow was temporarily occluded. G. L. Zhdanov [1] and the author showed that sarcolysin and TEM produce lesions of the bone marrow in the areas excluded from the circulation for 10-60 minutes. With longer periods of occlusion of the circulation of the bone marrow and administration of small, sublethal doses of sarcolysin, complete or almost complete protection of the excluded areas from the action of the drug is observed. However, after administration of TEM and exclusion of areas of the bone marrow from the circulation for 120 minutes, full protection cannot be obtained, even if small doses are given.

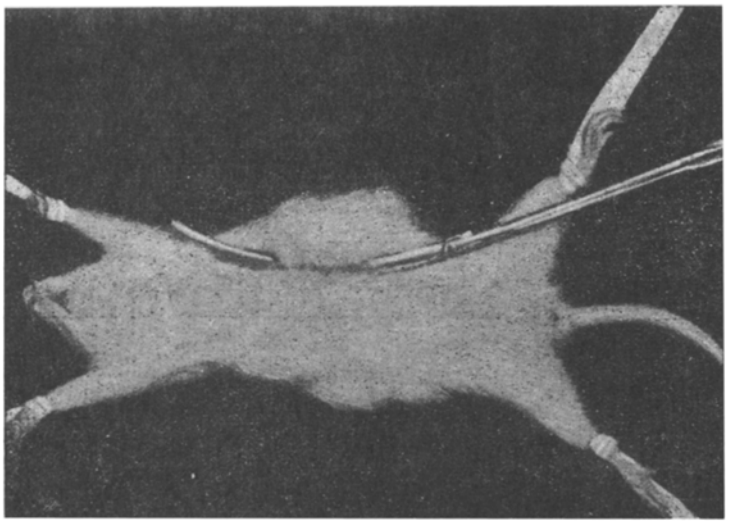

Fig. 1. An experimental rat in which the tumor on the left side is excluded from the circulation.

The impression was thus created that, despite the initially rapid fall in the concentration of sarcolysin and TEM, there is nevertheless a certain quantity of the preparation or of some other active agent, causing damage to the bone marrow, circulating in the blood stream for a considerable length of time.

In order to investigate the mechanism of the antitumor action of sarcolysin and TEM it was necessary to carry out experiments in which the blood supply of the tumors was cut off for different intervals of time, so that the importance of factors circulating in the blood could be ascertained. The first experiments in which sarcolysin was administered showed that the depression of growth of a tumor excluded from the circulation for 20 minutes is almost the same as that of a tumor which is not deprived of its blood supply [1].

We carried out experiments in which tumors of the sarcoma 45 type in rats were excluded from the circulation for various intervals of time and single or repeated injections of various doses of sarcolysin and TEM were given, and we also examined the possible effect of preliminary ischemia of the tumors.

EXPERIMENTAL METHOD

The investigation was carried out on 120 rats with sarcoma 45, i.e. with a tumor that is most susceptible to the action of sarcolysin and TEM. Suspensions of tumor cells were implanted subcutaneously at the same time in the right and left flank or in the right flank and the hindlimb. The experiments of administration of the preparations and exclusion of the circulation were performed on the 7th day after implantation, when the tumors had attained a measurable size. In order to cut off the blood supply of the tumor implanted in the flank, its skin "pedicle" was clamped with a pair of ordinary flexible, curved stomach forceps, as used in surgical practice (Fig. 1). To cut off the blood supply of a tumor implanted in the hind-limb, the peritoneal cavity was opened and the aorta and inferior vena cava clamped with Dieffenbach's forceps with rubber-covered blades. Under these circumstances the preparation was injected into the vena cava above the site of the clamp. The clamp was then removed and the abdomen closed and sutured. In addition, each series included a control group of animals which was not injected with the preparation. Each group consisted of 10 animals. In assessing the action of the preparation on the tumors, consideration was paid to the mean diameter of the "protected" and "unprotected" tumors, to the mean weight of these tumors in the control animals and in those receiving the preparations. The inhibition of growth of the tumors was calculated as a percentage by the formula: $T = \frac{M_0 - M_1}{M_0} \cdot 100$, where M_0 is the mean weight of the tumors in the control group and M_1 is the mean weight of the tumors in the experimental group.

The preparations were dissolved in physiological saline: sarcolysin at 60° and TEM at 20°.

The Action of Sarcolysin on the Growth of Sarcoma 45, Deprived of Its Blood Supply for Various Periods of Time (10 rats in each series)

Series No.	Tumors	Dose of sarcolysin		Time during which blood supply cut off (in min)	Weight of tumor (g)	Inhibition of growth of tumors, in %
		mg/kg	Number of injections			
C	Right	0	0	0	15.69	–
	Left	0	0	120	9.48	39
1	Right	15	1	0	0.68	95.6
	Left			20	0.8	94.4
2	Right	15	1	0	0.06	99.5
	Left			100* +20	0.2	98.6
3	Right	15	1	0	0.94	93.3
	Left			60	4.62	76.9
4	Right	15	1	0	0.77	95.0
	Left			120	14.92	4.9
5	Right	5	4	0	1.6	91.0
	Left			120	2.0	87.3
6	Right	4	4	0	2.91	81.4
	Left			120	3.7	76.4

* Preliminary ischemia of tumor before injection of the preparation.

EXPERIMENTAL RESULTS

The first series of experiments was carried out on 20 rats into which sarcolysin was injected intravenously and the aorta and inferior vena cava were clamped to cut off the blood supply of the tumor, implanted in the hindlimb, for 5, 10, 20 and 30 minutes. These experiments showed that exclusion of the tumor from the circulation for periods of up to 30 minutes during the administration of the preparation in a dose of 10 and 15 mg/kg body weight did not prevent cure of the tumor.

In the next series, experiments were performed on a large number of animals in which tumors were implanted in the right and left flanks. The blood supply of the tumors was cut off by the application of a clamp.

Exclusion of the tumor from the circulation for 20 minutes had no essential effect on the rate of cure of the tumor as a result of administration of sarcolysin (see Table, series 1). In series 2, the tumor was also deprived of its blood supply for 20 minutes during administration of the preparation, but it was subjected to preliminary ischemia for a period of 100 minutes. These experiments showed that preliminary ischemia of the tumor does not lower its susceptibility to the action of sarcolysin (see Table).

When the blood supply was cut off for 60 minutes some degree of protective action was observed: inhibition of growth of the tumor deprived of its blood supply amounted to 76.9%, whereas in the tumor not excluded from the circulation it was 94%.

A complete "protective" effect was observed when a single injection of a dose of sarcolysin of 15 mg/kg was given, only if the blood supply was cut off for 120 minutes. Under these circumstances the inhibition of growth of the tumor on the right side was 95%, and of that on the left side (blood supply cut off) – 4.9% only (see Table).

Hence it could be concluded that 2 hours after the injection of sarcolysin in a dose of 15 mg/kg, neither it nor other active products could be found in the blood in quantities capable of depressing the growth of sarcoma 45. However it must be pointed out that in the control animal, temporary ischemia of the tumor for 120 minutes resulted in inhibition of its growth by 39%, whereas in the experiment in which sarcolysin was injected

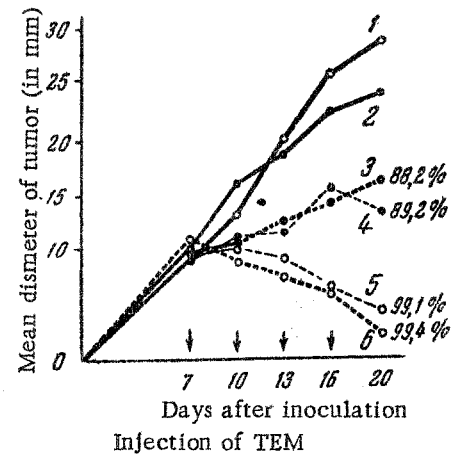

Fig. 2. The action of TEM on the growth of sarcoma 45 in rats when the blood supply of the tumor on the left side is cut off for 120 minutes.
1) Growth of the tumor (on the right) in the control rat; 2) growth of the tumor (on the left) in the control rat; 3) growth of the tumor (on the left) in an experimental rat (TEM 0.3 mg/kg); 4) growth of the tumor (on the left) in an experimental rat (TEM 0.4 mg/kg); 5) growth of the tumor (on the right) in an experimental rat (TEM 0.3 mg/kg); 6) growth of the tumor (on the right) in an experimental rat (TEM 0.4 mg/kg). The figures on the right indicate the inhibition of growth of the tumors in %.

and the blood supply cut off for the same length of time, no inhibition was apparent. Possibly in this case there was some stimulation of the growth of the tumor by very small doses of the preparation or by other active products remaining in the blood for longer than 120 minutes, which cut short the inhibiting effect of temporary deprivation of the blood supply.

To study further the problem, experiments were set up in which repeated injections of small doses of sarcolysin were given and the blood supply of the tumors was cut off. It could be assumed that a smaller dose of sarcolysin would disappear more rapidly from the blood stream, and therefore the protective effect would be obvious even when the blood supply was cut off for shorter periods of time. These experiments were carried out with four injections of sarcolysin in doses of 4 and 5 mg/kg at intervals of 72 hours, with correspondingly frequent interference with the blood supply to the tumors. However, the protective effect under these conditions was smaller than that after a single injection of sarcolysin in a dose of 15 mg/kg body weight (see Table, series 5 and 6).

In analysing these results it was essential to bear in mind that prolonged and repeated exclusion of the blood supply of a tumor may itself cause considerable inhibition of growth of the tumor. For instance, when the blood supply of the tumor was cut off on a single occasion for 120 minutes, it was 39%, but when cut off four times for 120 minutes, inhibition of growth of the tumor reached 58%.

Experiments in which the blood supply of a sarcoma 45 was cut off during administration of TEM were carried out with doses sufficient to cause strong inhibition of growth of this tumor. The preparation was injected intraperitoneally in a dose of 0.3 mg/kg and 0.4 mg/kg, four times at intervals of 72 hours, and immediately before the injections the clamp was applied to the tumor on the left side for 120 minutes. The rats were killed on the 20th day after inoculation. It was thus found that despite the exclusion of the blood supply of the tumor for 120 minutes, TEM caused depression of its growth. However the percentage inhibition of growth of the "protected" (left) tumors was lower, and for a dose of 0.3 mg/kg it amounted to 88.2, and for a dose of 0.4 mg/kg, 89.2, whereas in the "unprotected" (right) tumors it was 99.1 and 99.4 respectively (Fig. 2). These results show that exclusion of the tumors even for 2 hours from the general circulation, into which TEM is absorbed from its peritoneal cavity, did not completely protect them from the action of the preparation or of other active antitumor products circulating in the blood stream.

Experiments carried out with sarcolysin and TEM demonstrated the importance of an agent, circulating in the blood stream, in the process of cure of the tumor. The simplest explanation of this is the view that there is a gradual fall in the concentration of the preparations in the blood. However, in opposition to this view, the findings indicate a rapid fall in the concentration of sarcolysin and TEM in the blood in only the first few minutes after their administration, and in particular findings showing the disappearance of radioactivity from the blood after injection of labeled TEM [9]. The explanation of these different views evidently requires the assumption of either an action on the tumor by extremely small amounts of the preparations, circulating for a long time in the blood stream and gradually damaging the tumor, or the formation of certain additional products in the body associated with these preparations. It is perfectly obvious that investigations of the distribution and fate of antitumor preparations in the body would be of immense assistance in elucidating the mechanisms of the antitumor action of the alkylating agents. Such investigations are, however, still in their early stages at the present time.

It has been shown by studies of the distribution of labeled di-(2-chloroethyl)-amino DL-phenyl-β C^{14}-alanine (sarcolysin) that the total radioactivity of the blood, liver and tumor was at the same level. Under these circumstances the proteins had a higher activity than the nucleic acids [5]. Besides the absence of an additional label in the active chloroethyl group, an essential defect of this investigation was the fact that the comparative study of the distribution of radioactivity in the normal and tumor tissues was undertaken in the main at late periods (24-48 hours). Such results may evidently be of little assistance in analysing the mechanism of the antitumor action of sarcolysin, considering that the preparation injures the tumor mainly during the first two hours after injection into the body. At later periods a redistribution of the preparation may occur, practically unrelated to the process of damage to the tumor.

When TEM with a labeled triazine ring was administered, after 24 hours no selective accumulation of radioactivity could be detected in the tumor or any other tissues [8]. Preliminary results on the fate of TEM labeled with C^{14} in the ethylenimine group showed rapid disappearance of radioactivity from the blood. Unchanged TEM was not found in the urine, and only cyanuric acid, quite inactive against tumors, was excreted. In the course of 24 hours from 68-73% of the injected radioactivity was excreted in the urine. Chromatographic separation of the radioactive products showed, from preliminary rats, that at least 16 radioactive metabolites were formed in the urine [6, 9].

Evidently the use of the method of labeled compounds alone, and also of indirect biological tests such as, for instance, the temporary deprivation of the blood supply of organs and tumors susceptible to the action of antitumor preparations, are insufficient for the purpose of discovering the process of action of these preparations or their metabolites on normal and tumor tissues. One of the important steps in the study of the mechanisms of the antitumor action of the alkylating antitumor agents is further precise quantitative investigation of the concentrations of the preparations or of active products of their conversion in the tissues and fluids of the body, especially in early periods, studying at the same time the biological, and in particular the antitumor, action of these compounds.

SUMMARY

The author studied the effect of the temporary exclusion of the circulation from one of the two sarcomas "45" transplanted to rats on the antitumor effect of sarcolysin and of TET (a preparation known abroad as TEM). In single administration of sarcolysin (15 mg/kg) the exclusion of the circulation of the tumor for 20 minutes does not give any significant protective effect. In exclusion of the tumor from the bloodflow for 60 minutes a slight decrease of the inhibition of its growth is noted, while in its exclusion for 120 minutes the weight of the protected tumor shows almost no difference from the control tumor. Inhibition of the growth of unprotected tumor transplanted to the other side of the same rats reached 95%.

The protective effect was less pronounced in numerous administration of lower doses of sarcolysin with simultaneous exclusion of the circulation of the tumors.

Exclusion of the tumor circulation for 120 minutes in administration of 4 doses of TET (0.3 and 0.4 mg/kg) with the interval of 72 hours causes a slight decrease in the inhibition of the tumor growth.

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