ONCOLOGY

ON THE ANTITUMOR ACTIVITY OF CERTAIN DERIVATIVES OF SARCOLYSIN (DL-n-di-(2-CHLORETHYL) AMINOPHENYLALANINE)

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Among the synthetic di-2-chlorethylamines sarcolysin [DL-n-di-(2-chlorethyl)aminophenylalanine] has shown in experiments the strongest and widest antitumor activity [1, 2].

At present sarcolysin is employed clinically and has given positive results in treatment of certain malignant tumors and metastases [3].

However, effectiveness of the sarcolysin is limited only to certain human tumors so that there are valid reasons for working on the synthesis of new compounds for the treatment of other tumors.

With this goal in mind, we began a search for other antitumor compounds by modifying the sarcolysin formula, substituting either the carboxyl or amino groups. In addition to the practical bearing, the study of such compounds presents a great theoretical interest as it makes clear the meaning of the presence of the free carboxyl and amino radicals in antitumor sarcolysin activity.

With the above mentioned considerations in mind, we synthesized: 1) two compounds with substitution in the carboxyl group, ethyl and isopropylethers of DL-sarcolysin and 2) two compounds with substitution in the amino group, – DL-N-formylsarcolysin and DL-N-acetylsarcolysin.

EXPERIMENTAL METHODS

The ethylated ether of DL-sarcolysin was obtained by boiling a solution of sarcolysin for six hours in absolute ethanol saturated with dry HCl. This formed the dichlorhydrate ethyl ether of sarcolysin (melting point 174-177°), which hydrolyzed readily, becoming the monochlorhydrate with a melting point of 156-157° (in alcohol).

By an analogous procedure we obtained the monochlorhydrate isopropyl ether of DL-sarcolysin with a melting point of 187-189° (in alcohol).

In order to obtain DL-N-formylsarcolysin, the basic solution of sarcolysin in 96% formic acid was heated and acetic anhydride added drop by drop. The substance obtained had a melting point of 151-152° (in alcohol). At the same time, this compound was obtained by I. L. Knunyants, O. V. Kildisheva and N. E. Golubeva [4].

DL-N-acetylsarcolysin was synthesized by the action of acetic anhydride on sarcolysin dissolved in glacial acetic acid and then heated. The melting point is 152-153° (in alcohol).

EXPERIMENTAL RESULTS

Study of Biological Effects

The toxicity of the preparations was studied in adult rats by means of single intra-abdominal injections. Simultaneously, as standard controls, the toxicity of DL-sarcolysin was also determined.

The minimum lethal doses (LD_{50}) were determined for the preparations, these being the quantity causing the death of 50% of the animals. The results are given in Table 1.

TABLE 1

Dosages of the Preparations Producing Death in 50% of the Animals

Preparation	Dose in mg/kg
DL-sarcolysin	22
Ethyl ether of sarcolysin	17
Isopropyl ether	17
DL-N-formylsarcolysin	130
DL-N-acetylsarcolysin	90

When the animals were autopsied after the single injection had led to their deaths, regardless of the preparation used, there was observed macroscopically a shrinking of the spleen, lymph glands and the thymus, intestinal distension with fluid content and other signs characteristic of the action of the chlorethylamines.

Antitumor Activity

The antitumor activity of the preparations was studied on rats inoculated by the usual method with spindle cell sarcoma-45, done in the laboratory of experimental chemotherapy of the Institute of Experimental Pathology and Cancer Therapy. The antitumor activity of the preparations was compared with that of DL-sarcolysin.

There were three experimental modifications: 1) daily injections for 20 days; 2) a total of 7 injections at 72 hour intervals; 3) a single injection only.

In all instances, therapy commenced on the 7th day following tumor inoculation; animals were sacrificed on the 21st day after the beginning of therapy (on the 28th day following inoculation). Every 5 days the tumors were measured in three different directions and the average of the diameters was taken; its alterations being taken as an index of the dynamics of the preparation.

After being sacrificed, the animals were autopsied and the tumors stripped out, weighed and the percentage of growth inhibition determined.* In addition, the percentage of tumor resolution was determined separately. As was shown previously [1, 2], when sarcolysin dissolves sarcoma-45, there remains either a pigmented spot or a small soft node at the site of the former tumor. Histological examination has established that these nodes contain no tumor cells and disappear later after therapy has been terminated. We considered the tumor to have dissolved when such a nodule weighed 0.05 g or less. Nodes weighing over 0.05 g we counted as tumor tissue and we took their weight into account when determining the average weight of the tumors. Altogether we ran 4 series on 229 rats.

The results of the experiments are shown in Table 2.

As can be seen from Table 2, all four of the preparations studied have definite antitumor activity. So we have shown that antitumor activity is present both when the carboxyl group is substituted and also when the amino group of the phenylalanine in the sarcolysin molecule is substituted.

However, a comparison shows many biological differences in the actions of the preparations.

* Percentage of inhibition was calculated as usual from the following formula:

$$\frac{\text{average tumor weight in control} - \text{average weight in treated animals}}{\text{Average tumor weight in control animals}}$$

TABLE 2

Influence of Sarcolysin Derivatives Upon Tumor Growth

Expt. No.	Preparation	Number of animals			Dose in mg/kg	Interval between doses, hrs.	No. times prep'n. was introduced, mg/kg	Quantity of prep'n introduced, mg/kg	Average tumor weight		% inhibition	% dissolution
		controls	expt.	dying during expt.					controls	treated animals		
10	Sarcolysin	10	10	0	1.2	24	20	24	47.4	4.18	91.3	0
	Ethyl ether	10	10	0	1.2	24	20	24	47.4	8.5	82.0	0
	Isopropyl ether	10	10	0	1.2	24	20	24	47	10.83	77.2	0
11	N – formylsarcolysin	10	10	1	30	24	20	600	50.0	0.84	98.3	40
	"	10	10	0	10	24	20	200	50.0	13.75	61.2	0
13	Sarcolysin	10	10	0	1.5	24	20	30	53.7	4.50	91.6	20
	N – acetylsarcolysin	10	10	0	2.5	24	20	50	53.7	4.17	92.2	10
12	Sarcolysin	10	10	0	5.0	72	7	35	28.7	0.095	99.6	80
	Ethyl ether	10	10	0	5.0	72	7	35	28.7	0.148	99.5	60
	Isopropyl ether	10	10	0	5.0	72	7	35	28.7	0.0	100	100
	N – formylsarcolysin	10	10	1	30.0	72	7	210	28.7	2.45	91.4	20
	N – acetylsarcolysin	10	12	5	20.0	72	7	140	28.7	0.08	99.7	71
13	N – acetylsarcolysin	10	10	0	10.0	72	7	70	53.7	3.0	94.4	10
	Sarcolysin	10	10	1	6.0	72	7	42	53.7	0.0	100	100
12	"	10	10	0	15	Only once		15	28.7	0.12	99.5	70
	Ethyl ether	10	10	4	15			15	28.7	0.23	99.1	33
	Isopropyl ether	10	10	1	15			15	28.7	1.18	96.0	33
	N – formylsarcolysin	10	10	0	60			60	28.7	16.22	47.2	10
	N – acetylsarcolysin	10	10	0	20			20	28.7	0.04	99.8	70
13	N – acetylsarcolysin	10	9	0	30			30	53.7	0.75	98.6	66

When the carboxyl group is replaced by ethyl or isopropyl ether, both compounds resemble each other in their toxic and antitumor actions. Both the preparations are somewhat more toxic than sarcolysin and approach it in antitumor activity. When the amino group is substituted, the toxicity of the preparations drops markedly. Thus, for DL-N-formylsarcolysin the LD_{50} increases by a factor of 6 while for DL-N-acetylsarcolysin the toxicity factor rises fourfold when compared with DL-sarcolysin. The preparations differ from each other in their antitumor activity.

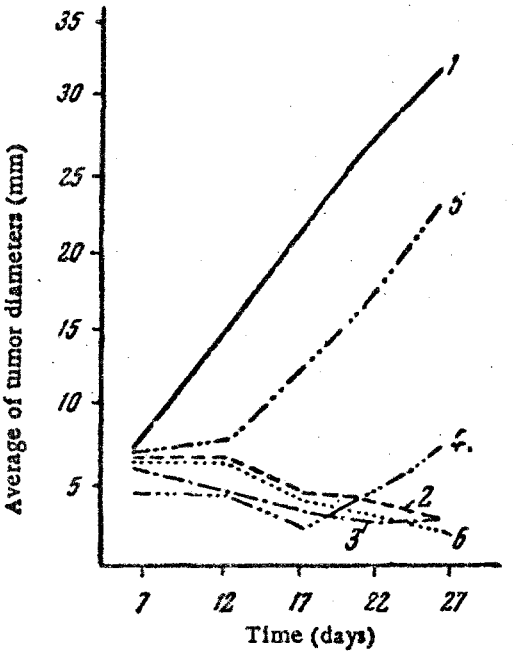

Fig. 1. Changes in the average diameters of the tumors, sarcoma-45, following a single injection of DL-sarcolysin (2), ethyl ether DL-sarcolysin (3), isopropyl ether DL-sarcolysin (4), DL-N-formylsarcolysin (5), and DL-N-acetyl-sarcolysin (6) all compared with the control curve (1).

The daily injection of DL-formylsarcolysin, whose dosage is 8 times that of sarcolysin, still gives a much weaker effect. In order to obtain a result approaching that obtained with sarcolysin, the dose has to be 25 times that of sarcolysin to produce death in some of the animals. The same occurs with other experimental modifications. Thus, in this preparation, the disparity between the toxic and therapeutic dosages is less than seen in sarcolysin and so this compound can have no practical value even when offering much theoretical interest.

On the other hand, DL-N-acetylsarcolysin produces an effect corresponding to that of sarcolysin in all experimental modifications, the dose exceeding that of sarcolysin only $1^1/_2$-2 fold. Thus, this preparation has a much greater disparity between the toxic and therapeutic dosage than seen with sarcolysin.

Eight rats in whom therapy with DL-acetylsarcolysin led to total tumor resorption, are still under continuing observation 2 months later. In this time no recurrences have been observed. This may mean that this compound will have practical value.

Final judgment as to the clinical applicability of the preparations will have to await a study of their effects on blood and animal organs, as well as their spectrum of antitumor activity.

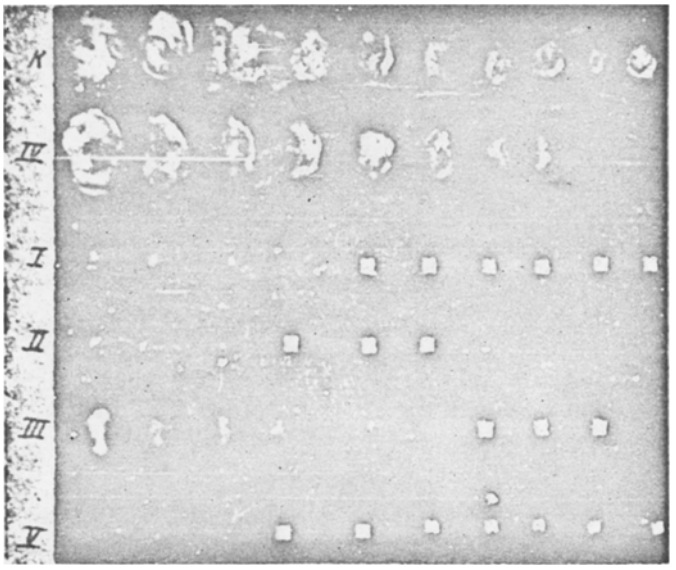

Fig. 2. Tumors (sarcoma-45) on the 21st day after a single injection of the preparations in the same dosages.
k-control. I) DL-sarcolysin; II) ethyl ether DL-sarcolysin; III) isopropyl ether DL-sarcolysin; IV) DL-N-formylsarcolysin; V) DL-N-acetylsarcolysin. White squares indicate rats in whom the tumors were fully resorbed.

The comparison of the toxic and antitumor activities of DL-N-formyl- and DL-N-acetylsarcolysin preparations leads us to take a great interest in examining replacement of the amino group on the sarcolysin with a longer alkylated chain. These studies are now in progress.

SUMMARY

Four modifications of sarcolysin were prepared. Two were substitutions in the carboxyl group: ethyl and isopropyl ethers of DL-sarcolysin; and two were substitutions in the amino group: DL-N-formylsarcolysin and DL-N-acetylsarcolysin. Animal tests were performed on rat sarcoma 45, a total of 229 rats being used to determine the LD_{50} and also the antitumor effect.

The carboxyl group substitutions were very similar in effects to sarcolysin itself. The amino substituted compounds were both less toxic and less effective against tumors. However, while in N-formylsarcolysin the difference between the toxic and therapeutic doses is less than in sarcolysin, the lethal dose of the N-acetylsarcolysin is about 4 times greater than the dose for sarcolysin itself. Thus, the therapeutic dose of this last modification has twice the safety of the corresponding sarcolysin dosage.

This last result has aroused interest in replacing the amino group with longer alkylated chains. Studies with such compounds are now in progress.

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