ONCOLOGY

THE ANTITUMOR ACTIVITY OF THE OPTIC ISOMERS OF SARCOLYSIN

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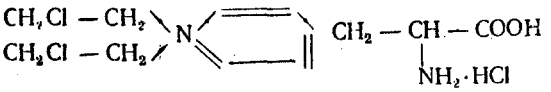
As has already been reported, the drug sarcolysin, which is DL-n-di-(2-chloroethyl) aminophenylalanine, was created according to the principle of attachment of active chemical groups to a metabolite which plays a leading part in the metabolism of tumor tissue [1]. In this particular case the chloroethylamine group was



combined with the unsubstituted amino acid DL-phenylalanine. Experimental research carried out on the compound thus obtained demonstrated its high antitumor acticity, which is more powerful than any of the other compounds of the chloroethylamines [3]. The presence of a natural component in the sarcolysin molecule called for investigation to ascertain the importance of its natural portion in the mechanism of the antitumor action of the compound.

TABLE 1

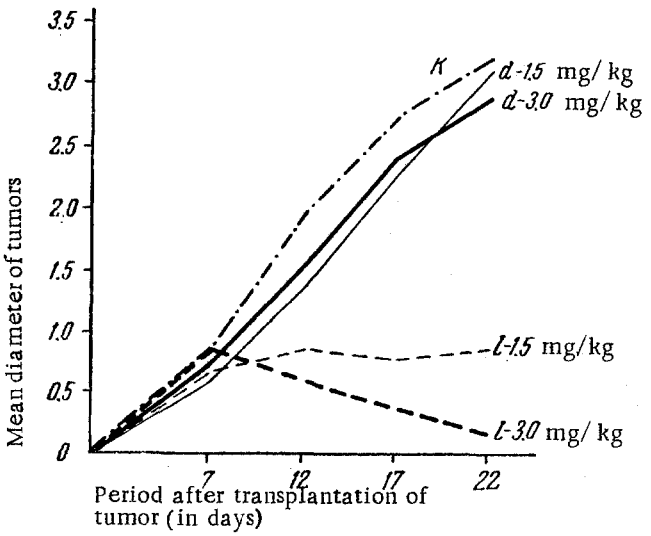
The Toxicity of Optical Isomers of Sarcolysin

Preparation	Dose, in mg/kg	No. of animals	No. of animals dying	% dying	Time of death in days
L-sarcolysin	25	10	9	90	4—6
	20	10	6	60	4—6
	15	10	3	30	4—6
	10	10	0	0	—
DL-sarcolysin	30	10	10	100	
	25	10	6	60	4—7
	20	10	4	40	4—7
	15	10	0	0	—
D-sarcolysin	35	10	10	100	3
	30	10	9	90	3
	25	10	6	60	3

TABLE 2

The Action of Optical Isomers of Sarcolysin on the Growth of Sarcoma 45

Experiment no.	Preparation	group	No. of animals	Dose in mg/kg (numerator and intervals between injections in hr. (denominator)	Mean wt. of tumor at the end of treatment (in g)	% retardation	% resolution
48	DL-sarcolysin	1	10	3/72	0.25	99.5	70
	as above	2	10	1.5/72	11.1	78.9	0
		3	10	0.75/72	24.7	53	0
	L-sarcolysin	4	10	3/72	0.09	99.8	70
	as above	5	10	1.5/72	1.95	96.2	30
		6	10	0.75/72	13.5	75	0
	Control	7	10	—	52.6	—	0
50	DL-sarcolysin	1	10	3/72	0.45	98.7	40
	as above	2	9	1.5/72	8.8	74.3	0
	L-sarcolysin	3	9	3/72	0.28	99.2	44
	as above	4	10	1.5/72	1.04	96.9	20
	D-sarcolysin	5	10	3/72	22.8	33.5	0
	as above	6	10	1.5/72	28.4	14.3	0
	Control	7	10	—	34.4	0	10



Growth of the tumors under the influence of optical isomers of sarcolysin. Treatment with D-sarcolysin (═══), treatment with L-sarcolysin (═ ═ ═), K — control.

The growth of tumors is known to be connected with intensive syntheses of protein, in the process of which amino acids are used up. It might therefore be suggested that sarcolysin, being a derivative of an unsubstituted amino acid, might enter into competetive relations with the natural unsubstituted amino acids and interfere with the syntheses of proteins, i. e. act according to the principle of an antimetabolite, especially as there are indications in the literature of the greater or lesser expression of antitumor action of the amino acid antagonists [2, 6, 9].

Another possible explanation was that the phenylalanine in the sarcolysin molecule may act as a "conductor" of the chemically active chloroethylamine group in the tumor tissue.

Sarcolysin, having one asymmetrical carbon atom in the alanine part of the molecule just like phenylalanine, may exist in the form of 2 optical isomers: the L-form of phenylalanine is natural, the D-form does not occur in nature.

If the hypotheses on which the creation of sarcolysin is based is correct, it would be expected that the laevo, natural isomer would possess the more pronounced biological activity.

In the papers by the British authors Bergel and Stock [4, 5] reference is made to Haddow's findings that the laevo isomer possesses greater antitumor activity. The different antitumor activity of the optic isomers is also mentioned in the paper by Koller and Veronesi [7].

In the laboratory of chemical syntheses of the Institute of Experimental Pathology and Therapy of Cancer of the AMN SSSR optical isomers of sarcolysin* have also been prepared, which enabled us to proceed to evalu-

* E. N. Shkodinskaia and O. S. Vasina (L-form) and I. S. Levi (D-form).

ate the role of the natural portion of the molecule of sarcolysin.

For this purpose in the present investigation a comparative study was undertaken of the toxicity and the antitumor activity of the D- and L-forms of sarcolysin.

EXPERIMENTAL METHOD

The investigation of the toxicity of the optical isomers of sarcolysin was carried out on 110 white rats weighing 100-120 g, and the investigation of the antitumor activity on 128 rats with a transplanted sarcoma 45.

The preparations were dissolved in physiological saline, heated preliminarily to 50-60° and injected intraperitoneally into the experimental animals. In the experiments to determine the toxity the compounds were injected once, but in the experimental treatment of sarcoma 45 they were injected repeatedly at intervals of 72 hours. Treatment continued for 21 days. Treatment began on the 7th-8th day after transplantation, i. e. while the tumors were measurable and weighed on the average of 0.7-0.8 g. The dosage of the compounds was calculated in milligrams of the dry compound per kg body weight of the animal.

In estimating the toxicity of the preparations we took into consideration the number of animals dying and the time of death. In estimating the antitumor activity attention was directed firstly, to the difference between the mean weight of the tumors in animals treated with the preparations and in the control animals, i. e. untreated (calculated as percentage retardation*), and secondly, to the changes in the growth of the tumors in animals of the same groups as determined by measurement of the tumors every fifth day (length, width, height), from which the mean diameter of the tumors was calculated.

The investigation of the toxicity and antitumor activity of the optical isomers of sarcolysin was carried out in comparison with the racemate.

EXPERIMENTAL RESULTS

In Table 1 are given the results of the experiments to determine the toxicity.

It can be seen from Table 1 that there is no sharp difference between the toxicity of L-sarcolysin and that of D-sarcolysin. The toxicity of the D-form is practically equal to that of the racemate L-form, being slightly greater than that of the latter. Thus the dose of DL-sarcolysin and D-sarcolysin which causes death of 60% of the animals is 25 mg/kg, whereas the corresponding dose of L-sarcolysin is 20 mg/kg.

The D- and L-forms are sharply distinguished from each other in their antitumor activity (Table 2): the activity of the L-sarcolysin in a dose of 3 mg/kg** is equal to the activity of the racemate and in smaller doses actually exceeds it; the D-form possesses only weak antitumor activity.

The difference in the antitumor activity of D- and L-sarcolysin is illustrated by the diagram in which are shown graphically the growth of the tumors and its changes under the influence of the optical isomers of sarcolysin in doses of 3 and 1.5 mg/kg, injected at intervals of 72 hours.

As can be seen from the Figure, the growth of the tumors during the action of D-sarcolysin on them proceeds almost parallel to the growth of the tumors in the control group of animals. Growth of the tumors under the action of L-sarcolysin is considerably retarded to an extent which depends directly on the dose.

The investigations carried out showed that the optical isomers of sarcolysin, which are quite similar in their chemical properties, differ in their biological activity.

The hypotheses put forward by Haddow, that the dextra isomer is more rapidly destroyed in the body as a result of the greater activity of D-amino acid oxidase, was not confirmed by experimental trial. Thus at the present time the most likely explanation of the difference in the antitumor activity of the optical isomers is their stereochemical structure.

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* The percentage retardation was calculated from the formula:

$$\frac{\text{Mean weight of tumors in control animals} - \text{Mean weight of tumors in experimental animals} \cdot 100}{\text{Mean weight of tumors in control animals}}$$

** This dose is slightly below the optimal dose for sarcolysin; the latter is 5 mg/kg when injected at intervals of 72 hours.

G. L. Zhdanov showed in our laboratory that the isomers of sarcolysin differ only slightly from each other in their action on the blood. They cause roughly the same degree of suppression of hemopoiesis, although the depression due to injection of the L-form is more lasting than that due to the D-form of sarcolysin.

On the basis of all the data it can be suggested that the slight differences in the toxicity and the action on the blood, in contrast to the great difference in antitumor activity, are explained by differences in the mechanism of action of sarcolysin on tumor and normal tissues. However this question demands further study. The fact that L-sarcolysin, possessing the natural configuration of the molecule, was found to be more active than the D-form of sarcolysin makes the hypotheses on which is based the creation of the antitumor preparation sarcolysin a probable one, and strengthens us in the opinion that the natural portion of the sarcolysin molecule plays a definite role in the mechanism of its antitumor action.

Further evidence in favor of this view is provided by the experiments which we performed to ascertain the influence of the structural analogues of sarcolysin on its antitumor activity.

Thus besides the racemate, L-sarcolysin can be recommended for clinical trial as an active antitumor preparation.

SUMMARY

Investigations were conducted on rats with transplantation of sarcoma 45. These experiments in coordination with literature data, demonstrated that optical isomers of the antitumor preparation sarcolysin, i. e. DL-p-di-(2-chloroethyl) aminophenylalanine, although being similar by their toxic properties, were different in their biological activity. Considerable antitumor activity of L-form of sarcolysin, possessing a natural configuration of the molecule, in difference from an unnatural L-form confirms the belief that metabolite in the sarcolysin molecule plays a definite role in the mechanism of the antitumor effect of this preparation.

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