FORMATION OF ANTIBODIES TO SARCOLYSINE AND THEIR EFFECT ON THE ANTITUMORAL ACTIVITY OF THE PREPARATION

(UDC 616-006-085.771.7-036.62-02 : 616.15-097 + 615.771.7-092.19 : 612.017.1)

A. I. Nikolaev, A. Kh. Akhmadieva, and G. F. Makarov

Uzbek Scientific Research Institute of Roentgenology, Radiology, and Oncology,
(Director—Professor D. M. Abdurasulov), Tashkent
(Presented by Member of the Academy of Medical Sciences, USSR,
N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 7,
pp. 95-98, July, 1965
Original article submitted January 9, 1964

Various chemical compounds, when introduced parenterally into the organism, may combine with its proteins, denature them, and convert them to foreign proteins [1]. It has also been found that when animals are injected with simple chemical compounds, and they combine with the serum, changes occur in the immunological specificity of the proteins, analogous to those which arise when the corresponding chemical radicals are introduced (by methods of organic synthesis) [4] into the protein molecule.

Antibodies formed in response to the introduction of toxic compounds may protect the animals from the action of these compounds [1].

Since sarcolysine, introduced into the organism, combines with proteins [5], the possibility of the formation of antibodies for neutralization of the preparation is not excluded. In oncology, this question is acquiring practical significance, since the appearance of antibodies neutralizing antitumoral preparations may substantially reduce their chemotherapeutic effect.

In this work we investigated the formation of antibodies to sarcolysine and studied their influence on the antitumoral activity of the preparation.

EXPERIMENTAL PROCEDURE

The investigations were conducted on rabbits and rats. In the first series of experiments we studied the significance of the introduced dose of sarcolysine in antibody formation in rabbits. For this purpose we tested two doses of the preparation: the maximum tolerable dose—1.5 mg/kg, and a third of it—0.5 mg/kg. The preparation was administered subcutaneously every other day, with a total of eight injections. On the tenth day after the last injection, the serum of the animals was tested for the presence of antibodies to sarcolysine in the reaction of precipitation with sarcolysine-casein. Reinjection of sarcolysine was conducted every 20 days.

Normal rabbit serum was used as the control in conducting the precipitation reaction.

Sarcolysine-casein was produced by the gradual addition of a 0.015% sarcolysine solution with mixing to a 1% casein solution at pH 8.0 (by analogy with the reaction of mustard gas with protein [7]), in equal volumes. One mole of protein accounts for approximately 10 moles of sarcolysine. The mixture was left overnight at room temperature. Sarcolysine-casein was precipitated and thoroughly washed with alcohol, then with alcohol with ether, and finally with ether. A dry grayish powder was obtained. An exact determination of the sarcolysine content in the casein is not compulsory for use of the preparation as a test antigen.

For the precipitation reaction, the antiserum was diluted with an equal volume of physiologic solution (1 :1), and the basic 1% solution was diluted 1 :2, 1 :4, 1 :16, etc. to obtain the test antigen.

In the second series of experiments, we investigated the influence of antibodies for sarcolysine on the antitumoral activity of the preparation.

TABLE 1. Influence of Dose of Sarcolysine on the Formation of Antisarcolysine

Rabbit No.	Dose of sarcolysine (in mg/kg)	Dilution of test antigen (sarcolysine-casein), giving a precipitation reaction with antisera			Rabbit No.	Dose of sarcolysine (in mg/kg)	Dilution of test antigen (sarcolysine-casein), giving a precipitation reaction with antisera		
		after immunization with sarcolysine	after first injection of sarcolysine	after second injection of sarcolysine			after immunization with sarcolysine	after first injection of sarcolysine	after second injection of sarcolysine
1	0.5	1 : 4	1 : 64	1 : 64	5	1.5	0	1 : 4	1 : 4
2	0.5	1 : 4	1 : 64	1 : 128	6	1.5	0	–	–
3	0.5	1 : 4	1 : 64	1 : 64	7	1.5	0	1 : 4	1 : 4
4	0.5	1 : 2	1 : 64	1 : 64	8	1.5	0	0	1 : 4

TABLE 2. Effect of Antisarcolysine Serum on the Antitumoral Activity of Sarcolysine

Conditions of experiment	No. of rats	Average weight of tumor (mg)	Inhibition of tumor growth (in %)	Weakening of antitumoral activity (in %)	t
Antisarcolysine serum + sarcolysine	5	4.20	71.5	28.5	16
Normal serum + sarcolysine	5	1.40	90.5	8.5	5.5
Physiologic solution + sarcolysine (first control)	6	0.36	97.5	–	–
Physiologic solution (second control)	9	14.72	–	–	–

The experiments were conducted on white rats with sarcoma 45. Five to six rats were used in each group.

For this purpose, on the 12th day after transplantation of sarcoma 45, the experimental animals were given intraperitoneal injections for a period of 18 days of antisarcolysine rabbit serum in doses of 1 ml daily and sarcolysine in doses of 5 mg/kg at 72-h intervals. The controls were animals that received sarcolysine and normal rabbit serum, as well as sarcolysine and physiologic solution, and only physiologic solution. The animals were killed on the 26th day after transplantation of the tumor.

The influence of antiserum on the antitumoral activity of sarcolysine was established according to the degree of weakening of its action (in percent), calculated at the end of the experiment on the basis of an investigation of the weight of the tumors in the control and experimental groups.

The data obtained were subjected to statistical treatment according to the small set method. The difference between the indices of the arithmetic means of the two variation series compared (t) was considered reliable at ≥ 3.

In the third series of experiments, we conducted active immunization of one group of rats with sarcolysine, and another with sarcolysine-casein followed by transplantation of sarcoma 45 and treatment of the animals with sarcolysine. The last group was taken as a supplementary control in order to eliminate the influence of preliminary administration of sarcolysine (toxic effect) on the result of the subsequent treatment, since it is known that the administration of antitumoral substances before transplantation stimulates tumor growth [6].

Immunization was carried out by the injection of sarcolysine (0.5 mg/kg) and sarcolysine-casein solutions (50 mg/kg) subcutaneously every other day, for a total of five injections. The presence of antibodies to the investigated substances in the serum of the animals was determined qualitatively by the reaction of precipitation with carcolysine-casein, diluted 1 : 4, in a depression slide [3] on the 12th day after the last injection. On the fifth day after the end of immunization, the rats received transplants of sarcoma 45; the course of treatment with sarcolysine was began on the 12th day of tumor growth. The preparation was administered intraperitoneally in doses of 5 mg/kg at 72 h intervals, for a total of four injections. The animals were killed on the 28th day of tumor growth.

TABLE 3. Effect of Immunization of Rats with Sarcolysine and Sarcolysine-Casein on the Antitumoral Activity of Sarcolysine

Immunization of rats (before transplantation of tumor)	No. of rats	Average weight of tumor (in g)	Inhibition of tumor growth (in %)	Weakening of antitumoral activity (in %)	Average weight of spleen (in g)	Average number of leukocytes	
						before experiment	after experiment
Sarcolysine (0.5 mg/kg)	6	4.58 t = 7.8	68.8 –	31.3 –	0.42 t = 4	14240	5310
Sarcolysine-casein (50 mg/kg)	5	3.20 t = 6.5	78.2 –	21.1 –	0.60 t = 7.6	12860	7170
First control (physiologic solution + sarcolysine)	6	0.36	97.5	–	0.22	15600	3500
Second control (physiologic solution)	9	14.72	–	–	1.20	14260	15620

The control animals received physiologic solution in place of the preliminary infections of the test substances. One group of rats was subjected to sarcolysine therapy, while the other was left untreated.

EXPERIMENTAL RESULTS

The results of the first experimental series are presented in Table 1.

From Table 1 it is evident that after immunization of the animals with sarcolysine in a dose of 0.5 mg/kg, the serum of the animals precipitated the test antigen, diluted 1 : 2 (rabbit No. 4) and 1 : 4 (rabbits Nos. 1-3). In reinjections, the antibody titer was increased to 1 : 64 (in one rabbit) to 1 : 28. In the animals that received sarcolysine injections in a dose of 1.5 mg/kg, antibodies were detected in the blood only after reinjection, and that in a small amount.

Consequently, the formation of antibodies to sarcolysine was more intense in those cases when the dose of the preparation was not toxic.

The data presented in Table 2, pertaining to the second series of experiments indicate that the administration of antisarcolysine serum lowers the antitumoral activity of sarcolysine. This is evidently explained by the fact that a certain portion of the preparation is blocked and neutralized by the introduced antiserum, which leads to a reduction of the free sarcolysine concentration in the tumor. A certain decrease in the inhibition of tumor growth after injections of sarcolysine and normal rabbit serum is probably explained by an increase in the metabolism in the organism and tumor in response to the introduction of a foreign protein.

From Table 3 is evident that the preliminary injection of the animals with sarcolysine and sarcolysine-casein substantially reduces the therapeutic effect of sarcolysine (by 21.1-31.3%). The lower degree of inhibition of tumor growth in experiments with preliminary administration of sarcolysine in comparison with experiments with sarcolysine-casein is probably due to the fact that the tumor reached a large size in these animals from the beginning of the course of treatment. Immunization of the rats with sarcolysine and sarcolysine-casein also prevented a reduction of the number of leukocytes and a decrease in the weight of the spleen.

Thus, our experiments indicate that the administration of sarcolysine induces the appearance of antibodies that neutralize it in the organism. These antibodies reduce the antitumoral activity of chloroethylamine. The data cited once again confirm the advisability of the use of massive doses of chemical preparations in the treatment of malignant tumors [2].

LITERATURE CITED

1. L. A. Zil'ber, Fundamentals of Immunology [in Russian], Moscow, (1958).
2. L. F. Larionov, Chemotherapy of Malignant Tumors [in Russian], Moscow, (1962).
3. A. I. Nikolaev, Byull. Eksper. Biol., No. 12. (1959), p. 79.
4. A. I. Nikolaev, Ibid., No. 1, (1960), p. 80.

5. M. A. Novikova, Summaries of Reports at the Eighth International Anticancer Congress [in Russian], Moscow, (1962), p. 309.
6. T. Kondo, Summaries of Reports at the Eighth International Anticancer Congress [Russian translation], Moscow, (1962), p. 295.
7. D. Northrop, M. Kunitz, and R. Herriott, Crystalline Enzymes [Russian translation], Moscow, (1950).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.