CHANGES IN THE DISTRIBUTION OF HYALURONIDASE IN ANIMALS AFFECTED BY TUMORS AS A RESULT OF THE ACTION OF AN ANTITUMOR SERUM

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In papers by several authors [2-5] there are reports that the development of a neoplasm in animals and man is accompanied by the appearance of the enzyme hyaluronidase in the blood and tissues. It has also been observed that hyaluronidase is a factor responsible for the infiltrating character of the growth of a neoplasm and for the degree of its metastasization.

The aim of our research was to investigate the appearance of hyaluronidase and its distribution in the organs during the development of a tumor, and to study the changes in this distribution under the influence of certain therapeutic factors.

METHOD

Rabbits were used as experimental animals. Preliminary experiments on healthy animals showed the absence of hyaluronidase in their organs and tissues. Further experiments were carried out on 59 rabbits, of which 44 were experimental and 15 control.

A 20% supspension of cells of a Brown-Pearce tumor in physiological saline was inoculated into the left sex gland of all the rabbits. On the 12th day, when growth of the tumor could be observed in the primary focus, together with involvement of the internal organs, injections of Prof. V. A. Chepurin's [5] anticancer serum were begun, in a dose of 0.3 ml/kg body weight. The serum was injected subcutaneously twice a week. At different periods of treatment animals were sacrificed and their organs and tissues investigated for their hyaluronidase content by the McCline – Smirnova method. Extracts of the various organs were mixed in definite proportions with hyaluronic acid and incubated for 30 minutes. After cooling, 15% acetic acid was added, and the reaction was regarded as positive (indicating the presence of hyaluronidase in the organs) if no mucin clot was formed in the precipitate. If a clot appeared, the reaction was considered to be negative. In order to denote involvement of the organs with metastases and the presence therein of hyaluronidase, certain conventional signs were adopted (see Table 1).

At the same time blood films were periodically taken from the animals for the differential white cell count, and blood was also taken for determination of the total white cell count.

RESULTS

It was shown that the largest quantity of hyaluronidase was present in the organs and tissues of the animals of the control group, which received no anticancer serum. These animals also showed a total leukocytosis, and the leukocyte formula revealed a neutrophilic leukocytosis and lymphocytopenia. The animals of the control

TABLE 1

Degree of Metastasization in Organs and Their Content of Hyaluronidase in Rabbits Not Receiving Anticancer Serum (Controls)

Rabbit No.	wt. (in g) before inoculation	wt. (in g) at the moment of death	Time of death (sacrifice) of animal	Material investigated: heart	lung	diaphragm	omentum	gastric mucosa	spleen	liver	mesentery	adrenal	kidney	peritoneum	bladder	sex gland	blood serum
1	2200	1800	13th day (sacrificed)	++	++	++	+++	0	0	++++	+++	0	+++	++++	++	++++	0
				2	2	2	3	0	0	5	4	0	5	5	2	4	5
2	2220	1850	13th day (sacrificed)	++	++	++	+++	0	0	++++	+++	0	+++	++++	++	++++	0
				2	2	2	3	0	0	5	4	0	5	5	2	4	5
3	2150	1900	14th day (sacrificed)	++	+++	+++	+++	0	0	+++	++	0	++++	++	++	+++	0
				2	3	3	3	0	0	5	3	0	4	2	2	3	5
4	2020	1500	14th day (sacrificed)	0	++	0	0	0	0	++++	++	0	+++	+++	0	++	0
				0	2	0	0	0	0	5	3	0	4	3	0	2	5
5	1900	1555	15th day (died)	0	++	0	0	0	0	++++	+++	0	+++	0	0	+++	0
				0	2	0	0	0	0	5	3	0	4	0	0	3	5
6	1850	1600	15th day (died)	0	0	0	++	0	0	+++	++	0	+++	++	0	+++	0
				0	0	0	3	0	0	5	3	0	4	2	0	3	5
7	2150	1950	16th day (died)	0	++	++	+++	0	0	++++	0	0	+++	++	0	++++	0
				0	2	0	4	0	0	5	0	0	4	2	0	5	0
8	1950	1750	16th day (died)	++	++	++	+++	0	0	++++	+++	0	++	0	0	+++	0
				2	2	2	4	0	0	5	3	0	3	0	0	4	5
9	1800	1600	17th day (sacrificed)	++	++	++	+++	0	0	++++	++	0	+++	++	++	+++	0
				2	2	2	4	0	0	5	2	0	4	2	2	4	5
10	1900	1750	17th day (sacrificed)	++	0	++	+++	0	0	++++	++	0	+++	+++	0	++++	0
				2	0	2	4	0	0	5	2	0	4	2	0	5	5

TABLE 1 (continued)

Rabbit No.	wt. (in g) before inoculation	wt. (in g) at the moment of death	Time of death (sacrifice) of animal	Material investigated: heart	lung	diaphragm	omentum	gastric mucosa	spleen	liver	mesentery	adrenal	kidney	peritoneum	bladder	sex gland	blood serum
11	2000	1800	18th day (died)	++	++	++	+++	0	0	++++	++	0	++	+++	0	+++	0
				2	2	4	4	0	0	5	2	0	2	3	0	4	5
12	2100	1850	20th day (died)	++	0	+++	+++	0	0	++++	+++	0	+++	+++	0	++++	0
				2	0	3	4	0	0	5	3	0	4	3	0	5	5
13	2050	1600	25th day (died)	0	++	++++	++++	0	0	++++	++	0	+++	++	0	0	0
				2	0	5	5	0	0	5	2	0	4	2	0	0	5
14	2120	1550	29th day (died)	0	++	+++	++++	0	0	++++	++	0	+++	++	0	0	0
				0	2	4	5	0	0	5	2	0	3	2	0	0	5
15	1960	1500	30th day (sacrificed)	+++	++	+++	++++	0	0	++++	0	0	++	++	0	++++	0
				2	2	3	5	0	0	5	0	0	3	2	0	4	5

Legend: + few metastatic nodes present; ++ groups of nodes present; +++ considerable number of metastases; ++++ whole organ affected. Hyaluronidase content of organs: 0) absent; 1) weakly positive reaction; 2) positive in the first two tubes; 3) in the first three; 4) in the first four; 5) strongly positive reaction, hyaluronidase present in all five tubes.

TABLE 2

Degree of Metastasization of Organs and Their Content of Hyaluronidase in Rabbits Receiving Anticancer Serum (Experimental Animals)

Rabbit No.	wt.(in g) before inoculation	wt.(in g) at the moment of death	Time between inoculation tumor + start of treat.(days)	No. of injections of serum	Time of sacrifice of animal (days after begin. of treat.)	Material investigated: heart	lung	dia-phragm	omentum	gastric serosa	spleen	liver	mesentery	adrenal	kidney	peri-toneum	bladder	sex gland	blood serum
1	2 200	1 650	12	1	15th	0	++	++	++	0	0	++++	++	0	+++	++		++++	0
						0	2	3	3	0	0	5	2	0	4	2		4	5
2	2 150	1 750	12	1	17th	0	++	++	+++	0	0	++++	++	0	+++	++		++++	0
						0	2	2	3	0	0	5	2	0	4	2		4	5
3	2 200	1 800	12	1	18th	0	++	+++	0	0	0	+++	++	0	++	0		++	0
						0	2	2	0	0	0	4	2	0	3	0		2	5
4	1 980	1 550	12	2	19th	0	++	++	0	0	0	+++	++	0	++	0		++	0
						0	2	2	0	0	0	4	2	0	2	0		2	4
5	1 950	1 800	12	2	19th	0	++	++	0	0	0	+++	++	0	++	0		++	0
						0	2	2	0	0	0	4	2	0	2	0		2	4
6	2 000	1 850	12	2	20th	0	0	++	0	0	0	+++	++	0	++	0		++	0
						0	0	2	0	0	0	3	2	0	2	0		2	4
7	2 100	1 900	12	2	20th	0	0	0	0	0	0	+++	++	0	++	0		++	0
						0	0	0	0	0	0	3	2	0	2	0		2	4
8	2 180	2 100	12	3	21st	0	0	0	0	0	0	++	0	0	++	0		+++	0
						0	0	0	0	0	0	2	0	0	2	0		4	4
9	2 140	2 000	12	3	22nd	0	0	0	0	0	0	0	0	0	+++	++		+++	0
						0	0	0	0	0	0	0	0	0	4	3		4	4
10	2 020	2 000	12	4	23rd	0	0	0	0	0	0	++	0	0	++	++		+++	0
						0	0	0	0	0	0	2	0	0	3	3		5	4

TABLE 2 (continued)

Rabbit No.	wt (in g) before inoculation	wt (in g) at the moment of death	Time betw. inocul. tumor and start of treat. (days)	No. of injections of serum	Time of sacrifice of animal (days after begin. of treat.)	Material investigated: heart	lung	diaphragm	omentum	gastric serosa	spleen	liver	mesentery	adrenal	kidney	peritoneum	bladder	sex gland	blood serum
11	2 000	2 000	12	4	23rd	0 / 0	0 / 0	0 / 0	++ / 2	0 / 0	0 / 0	++ / 2	0 / 0	0 / 0	++ / 0	++ / 3		+++ / 3	0 / 4
12	2 150	2 140	12	4	24th	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	++ / 2	0 / 0	0 / 0	++ / 2	+ / 0		++ / 3	0 / 4
13	2 100	2 120	12	5	25th	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	++ / 2	0 / 0	0 / 0	0 / 0		++ / 2	0 / 3
14	2 200	2 180	12	6	29th	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	++ / 2		++ / 3	0 / 2
15	2 300	2 250	12		30th	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	++ / 2	0 / 0	0 / 0	0 / 0	0 / 2		+++ / 3	0 / 2

group lost 30-40% of their original weight (Table 1). In the animals of the experimental group the largest quantity of hyaluronidase was observed in those cases in which the animals received only one or two injections of anticancer serum. As the number of injections of anticancer serum increased in these animals, the metastases disappeared from their organs and their hyaluronidase content became smaller until it reached a minimum value, and in animals which received 5-6 injections of the therapeutic serum the hyaluronidase disappeared altogether (Table 2). The duration of survival of these animals increased and they ceased to lose weight. Study of their blood picture showed a decrease in the original leukocytosis, which gave way in the course of treatment to a reduction in the total white cell count but in the leukocyte formula the neutrophilic leukocytosis was gradually replaced by a lymphocytosis.

SUMMARY

An attempt was made in this work to trace the dynamics of hyaluronidase distribution in the body during the development of a Brown-Pearce tumor, and the changes in the hyaluronidase level in the organs following administration of anticancer serum. Preliminary experiments on healthy animals demonstrated the absence of hyaluronidase in their blood and organ tissues. With pronounced metastases, a marked positive reaction to the presence of hyaluronidase was seen in the blood serum and extracts of various organs (liver, kidneys, gonads, etc.) of experimental animals. With gradual resorption of the tumor nodes, resulting from the action of V. A. Chepurin's cancer antiserum, the amount of hyaluronidase in the organs and tissues declined; after the resolution of the nodes, hyaluronidase disappeared completely. As time went on some changes were also noted in the other indices in connection with the cancer antiserum administration. The blood picture showed a tendency to normalization, and the loss of weight, which was initially marked, was arrested.

LITERATURE CITED

1. S. M. Bychkov, in: Progress in Biological Chemistry [in Russian] (Moscow, 1950) Vol. 1, p. 456.
2. E. Ya. Geiman, Uspekhi Sov. Biol. 23, 3, 324 (1947).
3. F. A. Gluzman, Med. Zhur. Ukrain. 20, 63 (1950).
4. L. G. Smirnova, in: Advances in Modern Biochemistry [in Russian] (Moscow, 1947) Vol. 1, p. 302.
5. V. A. Chepurin, Byull. Éksp. Biol. Med. 5, 99 (1960).*
6. G. N. Chistovich, Zhur. Mikrobiol., Épidemiol. i Immunobiol., 9, 44 (1947).

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