EFFECT OF HYALURONIDASE, HYALURONIC ACID, AND SOME OTHER SUBSTANCES ON POSTRADIATIONAL EXPERIMENTAL BACTERIEMIA

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Data in the literature [1-7, 9-19, 22, 24-27] indicate the important role of hyaluronidase and hyaluronic acid in radiation pathology. The object of the present study was to investigate the effect of x-rays on the activity of hyaluronic acid and its specific enzyme – hyaluronidase – in vitro, to investigate the dynamics of the bacteriemia in albino mice and rabbits exposed to the combined action of x-rays and hyaluronidase, and to examine the effectiveness of a scheme of treatment of radiation sickness developed by the authors.

EXPERIMENTAL METHOD

Experiments in vitro. Hyaluronidase and hyaluronic acid in sealed ampules were irradiated on the RUM-11 apparatus with a voltage of 187 kV, current 15 mA, focal distance 18 cm, field of irradiation 10 × 10 cm, dose rate 600 R/min, without filters. The doses of x rays were 100, 200, 300, 500, 1000, 1500, 2000, 4000, 6000, 8000, 12,000, 16,000, 24,000, 32,000, 48,000, 64,000, and 96,000 R. Before and immediately after irradiation, the activity of both preparations was determined in the McClean–Smirnova reaction, with the aid of a viscosimeter and mixing chamber for leukocytes.

Experiments in vivo. The animals were irradiated once on the RUM-11 apparatus at a voltage of 187 kV, current 15 mA, filters 0.5 mm Cu + 2 mm Al, skin-focus distance 60 cm (for rabbits) and 40 cm (for mice), and a dose rate of 15 or 48 R/min. In experiments on 200 albino mice and 60 rabbits, the bacteriemia was studied after irradiation (400, 450, 650 R) and administration of the enzyme lidase. Lidase was injected intraperitoneally into the mice in a dose of 16 units per injection once daily for one week in the postradiational period. Two doses of the enzyme were tested on the rabbits. The animals irradiated in a dose of 400 R received lidase in a dose of 64 units intravenously immediately after irradiation, 24 and 48 h after irradiation, and on the 7th and 14th days of radiation sickness. The rabbits of the other group (650 R) were injected with lidase in a dose of 16 units per intravenous injection immediately after irradiation and on the 10th day of radiation sickness. For the animals of all the experimental groups a corresponding control was provided. The rabbits were under observation for one month.

Blood was taken from the marginal vein of the ear and seeded on a sucrose agar plate. The seedings were incubated for 48 h, and the growing colonies were counted.

In the next section of the observations the dose of radiation used was 600 R. The postradiational bacteriemia was studied in 600 albino mice injected with vitreous body (VB) and the other substances envisaged in the authors' scheme after irradiation.

Treatment of the animals began on the day of irradiation. The therapeutic agents were administered parenterally (intraperitoneally) and orally. The following combinations of preparations were tested: on the animals of group 1 – VB, adrenalin, chlortetracycline, and vikasol (a synthetic vitamin-K preparation); group 2 – VB, adrenalin, and chlortetracycline; group 3 – VB, adrenalin, and vikasol; group 4 – VB, chlortetracycline, and vikasol; group 5 – adrenalin, chlortetracycline, and vikasol; group 6 – chlortetracycline

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Table 1. Bacteriemia in Rabbits Exposed to the Combined Action of X-rays and the Enzyme Lidase

No. of rabbits	Experimental conditions	Times of observation											
		1 h	3 h	6 h	10h	24 h	2 days	7days	14 days	17 days	25 days	30 days
10	400 R+lidase (64 units)	0/10	0/10	2/10	5/10	9/10	9/10	9/10	9/9	9/9	6/6	4/5
10	400 R	0/10	0/10	0/10	0/10	0/10	0/10	8/10	9/10	10/10	0/10	0/9
10	Lidase (64 units)	0/10	0/10	0/10	0/10	5/10	7/10	3/10	6/8	0/8	0/8	0/7
							3days	5days	10 days		20 days		
10	650 R+lidase (16 units)	0/10	0/10	7/10	9/10	9/9	9/9	9/9	8/9	6/6	—	—	—
10	650 R	0/10	0/10	0/10	0/10	0/10	5/7	7/10	9/9	9/9	5/9	1/1	—
10	Lidase (16 units)	0/10	0/10	0/10	0/10	3/10	4/10	0/10	6/10	0/10	0/10	0/10	0/8

Note. Numerator – number of animals in which bacteriemia was found; denominator – number of rabbits taken in experiment; 0 – absence of bacteriemia; – investigation not carried out because of death of animals.

and vikasol; group 7 – adrenalin and vikasol; group 8 – chlortetracycline and adrenalin; group 9 – VB and chlortetracycline; group 10 – VB and adrenalin; group 11 – VB and vikasol; group 12 – controls receiving x-ray irradiation (600 R only). According to data in the literature [4, 5, 8, 20, 21, 21], one of the most effective antibiotics for the treatment of the infectious complications of radiation sickness is chlortetracycline, which was used in the present experiments.

Blood was taken from the tail vein of the mice and seeded on sucrose agar plates. The investigations were carried out after 24 h and on the 3rd, 5th, 10th, 20th, and 30th days of radiation sickness. At the same time, the leukocyte count, the changes in body weight, and the general condition of the animals were studied. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The experimental results showed that x-rays have only a slight action on hyaluronidase activity; yet, at the same time, they had a very marked effect on the hyaluronic acid (after irradiation in doses of 24,000-96,000 R, total destruction took place). These results, obtained in the McClean–Smirnova reaction, were confirmed by the results of the viscosimetric tests and tests of the hyaluronic acid activity by means of a leukocyte mixing chamber. During determination of the viscosity of the preparation in a leukocyte mixing chamber, it was found that its viscosity fell from 23.5 sec (before irradiation) to 4.5 sec (96,000 R); when a type VK-4 viscosimeter was used, the viscosity of the hyaluronic acid also fell from 7 (before irradiation) to 0.65 (64,000 and 96,000 R).

Analysis of the results of the experiments in vivo shows that in the mice exposed to the combined action of irradiation and lidase, the bacteriemia was incomparably more severe in character than in the animals irradiated only (see table).

The bacteriemia began in the irradiated rabbits 6-10 h after administration of the enzyme. In the rabbits exposed to irradiation only it did not appear until the 3rd (650 R) or the 10th (400 R) day after irradiation and had subsided by the end of the investigation. The microorganisms entered the blood stream of the unirradiated rabbits receiving lidase 24 h after injection of the enzyme. Clearance of the microorganisms from the blood took place by the 14th-17th day of radiation sickness. Consequently, the combined action of x rays and the enzyme lidase considerably increased the severity of the radiation sickness.

The results of the investigations of the combined treatment of acute radiation sickness showed that the most effective of the eleven methods of treatment was the first (VB + chlortetracycline + adrenalin +

vikasol): in this case the onset of the disease was later, and the bacteriemia (present in 43% of cases) began after the 10th day after irradiation (64% of cases in the control). The proportion of surviving mice in this group on the 30th day of the investigation was 90%, compared with 6% in the control. On the whole, the most effective combinations were Nos. 1, 4, 9, 2, and 3, namely, those in which VB was used. These results are statistically significant.

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