SOME PROPERTIES OF LOW-MOLECULAR-WEIGHT TETANUS ANTITOXIN

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UDC 615.373.7:576.851.551

After intravenous injection of tetanus antitoxin obtained by tryptic digestion of "Diaferm-3" horse immunoglobulin, purification, and concentration of the active fragments, the antitoxin was excreted by rabbits 3 times faster than after injection of the original "Diaferm-3" antitoxin. After injection of the fragmented antitoxin its excretion continued until the 6th days, whereas after injection of "Diaferm-3" antitoxin its excretion continued until the 19th day; in the first case much less antitoxin was excreted than in the second (2% and 3.5% respectively). In both cases the antitoxin excreted in the urine consisted of monovalent Fab' fragments which caused delay of precipitation in the cross reaction in agar gel between tetanus toxoid and antitetanus serum. The Fab' fragment obtained by this method possessed anaphylactogenic properties.
KEY WORDS: *low-molecular-weight antibodies; tetanus; tetanus antitoxin.*

The study of the circulation of immune serum in passively immunized animals has shown three phases of elimination of heterogeneic proteins [15]. The first (equalizing) phase lasts 2-3 days, during which 30-40% of the protein is eliminated from the blood stream, and it depends on the distribution of the heterogeneic protein between the blood and tissues. The second phase, that of logarithmic elimination, depends on the intensity of metabolism of the protein, and it therefore differs in different species of animals [9]. The third phase has been called the phase of immune elimination and it depends on the appearance of antibodies against the heterogeneic proteins and is characterized by a sharp decrease in their blood levels [3, 10, 11]. If homologous proteins are injected, this last phase is ill-defined [1].

TABLE 1. Anaphylactogenic Properties of Antitoxins

Antitoxin and sensitizing dose	Antitoxin and reacting dose	Number of animals	Reaction				
			lethal	severe	average	mild	absent
Fab' fragment (10 mg)	Fab' fragment (100 mg)	21	2	3	5	7	4
Ditto	"Diaferm-3" (100 mg)	21	1	3	7	6	4
"Diaferm-3" (10 mg)	Fab' fragment (100 mg)	21	1	2	5	8	5
Ditto	"Diaferm-3" (100 mg)	21	2	4	7	5	3

In passively immunized animals heterogeneic antibodies appear after a certain time interval in the urine as low-molecular-weight fragments similar to those formed by digestion of immune globulins by proteolytic enzymes [2, 5, 12].

According to some workers [14], the rapidity of elimination of antibodies depends on the size of their molecules. For instance, bovine serum albumin (BSA) circulated in the blood of rabbits for 14 days. Low-molecular-weight fragments (Fab' and Fd) of BSA-competent antibodies circulated in rabbits for 2-3 days; if, however, preliminary injections of BSA were given to the animals, the immune fragments of antibodies formed complexes with them which circulated in the blood stream of the rabbits for 12-14 days.

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L'vov Medical Institute. Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 3, pp. 331-333, March, 1976. Original article submitted June 12, 1975.

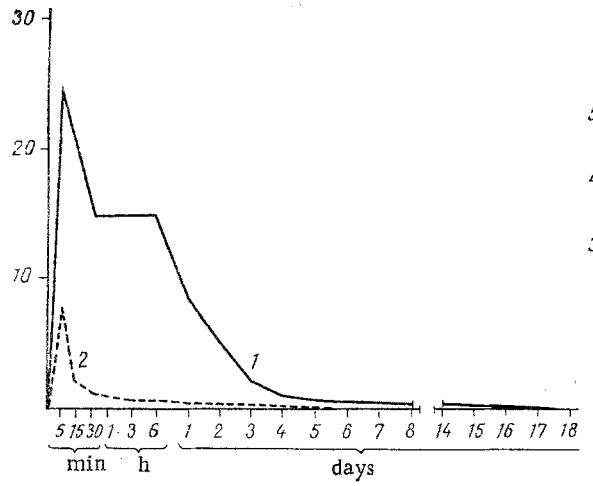

Fig. 1

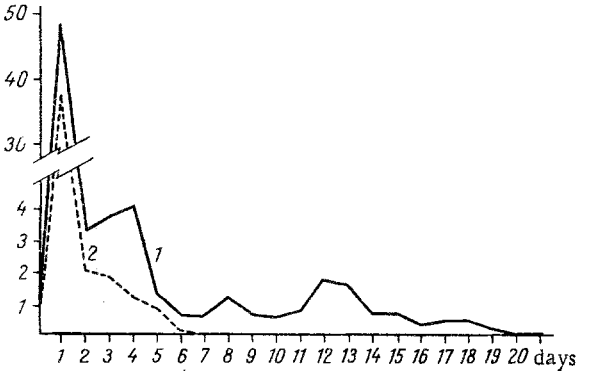

Fig. 2

Fig. 1. Dynamics of circulation of antitoxins in rabbits' blood stream: 1) "Diaferm-3"; 2) low-molecular-weight antitoxin. Ordinate, titer of antitoxin (in i.u./ml); abscissa, time of testing.

Fig. 2. Dynamics of elimination of antitoxins in urine. Legend as in Fig. 1.

The "Diaferm-3" antitetanus serum, the molecule of which consists of two undissociated Fab' fragments [$F(ab')_2$], induces anaphylactic reactions of immediate type, sometimes terminating in death [6-8].

The object of this investigation was to study the dynamics of elimination of the low-molecular-weight and ordinary "Diaferm-3" antitoxin in rabbits and also their sensitizing properties.

EXPERIMENTAL METHOD

"Diaferm-3" tetanus antitoxin, produced by the I. I. Mechnikov Moscow Institute of Vaccines and Sera, was subjected to tryptic porteolysis with an enzyme/substrate ratio of 1:50 and at pH 8.5 and a temperature of 37°C for 24 h in the presence of 0.02 M cysteine. The coagulated proteins were removed by centrifugation and the supernatant was fractionated on Sephadex G-100; the fractions were collected by means of a KhKOV-1 automatic collector. Fractions containing low-molecular-weight antitoxin were identified in the precipitation delay test in 1% agar gel between tetanus toxoid and antitetanus serum. These fractions induced specific delay, but did not themselves flocculate with toxoid. They were pooled, poured into a cellophane bag, and concentrated by dialysis against polyethylene glycol with a molecular weight of 40,000 daltons in the cold to 10% protein. The specific activity of the low-molecular-weight antitoxin was 12-15 i.u./mg protein, compared with a specific activity of the "Diaferm-3" antitoxin of 8-11 i.u./mg protein.

The dynamics of circulation of the antitoxins was studied in experiments on 23 adult rabbits of both sexes, after intravenous injection of antitoxins in a dose of 1000 i.u./kg into each animal, by determination of the blood antitoxin titer.

To study elimination of the antitoxins from the animals by the kidneys, the 24-h sample of urine was collected, the antitoxin titer was determined, and the quantity of antitoxin excreted during the 24-h period was calculated.

The properties of the antitoxin excreted in the urine, as regards flocculating and delaying flocculation, were studied after concentration of the urine in the crossed precipitation delay test in 1% agar gel. Antibodies against horse serum proteins or their fragments were detected by the ring precipitation test.

The anaphylactogenic properties of the antitoxin were studied in noninbred guinea pigs weighing 250-300 g, 10 mg antitoxin protein being used as the sensitizing dose and 100 mg protein as the reacting dose, injected intravenously 4-6 weeks after sensitization.

EXPERIMENTAL RESULTS

The dynamics of circulation of the antitoxins in the rabbits' blood stream is illustrated in Fig. 1.

As Fig. 1 shows, 5 min after injection the titer of the low-molecular-weight antitoxin was 3 times lower than that of the "Diaferm-3" antitoxin. After 15 min it was 8-10 times lower, after 3 h it was 30-40 times lower, after 2 days 60-70 times lower, and on the 7th day this antitoxin had disappeared completely from the blood, whereas the titer of "Diaferm-3" antitoxin on the 7th day was between 0.3 and 0.7 i.u./ml, and it still remained in diminishing quantities until the 18th day.

The result of elimination of the antitoxins from the rabbits in the urine are shown in Fig. 2.

The tests showed that "Diaferm-3" antitoxin could be detected in the rabbits' urine until the 19th day in decreasing concentrations. On the 8th and 12th-13th days the excretion of antitoxin was somewhat increased, evidently because of the production of antibodies against horse serum proteins, which began to appear in the rabbits' blood after the 8th day.

The fragmented antitoxin (Fab' fragments) was detected in the rabbits' urine for only 6 days; its excretion thus began and, indeed, ended before the phase of immune elimination had begun. No antibodies against horse serum proteins or their fragments could be detected at any time during this period in the rabbits' blood. The fact will be noted that considerably less of the low-molecular-weight antitoxin was excreted in the urine than of the "Diaferm-3" antitoxin (43.37 and 70.76 i.u. respectively). The molecules of the low-molecular-weight antitoxin were evidently destroyed much more rapidly in the rabbits. The quantity of antitoxin excreted in the urine, however, was only between 2 and 3.5% of the injected dose. In both cases antitoxin found in the urine of the animals consisted of monovalent fragments capable of inducing delay of precipitation between tetanus toxoid and antitetanus serum.

The results of a study of the anaphylactogenic properties of the low-molecular-weight antitoxin are given in Table 1.

They show that the low-molecular-weight antitoxin possessed sensitizing properties to almost the same degree as "Diaferm-3" antitoxin. Similar results were obtained by Schultze et al. [13].

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