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POTENCY TRIALS OF LOW-MOLECULAR-WEIGHT TETANUS ANTITOXIN

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There is much evidence in the literature to show that tetanus antitoxin can neutralize free tetanus toxin circulating in the blood stream. At the same time it has been shown that tetanus toxin, when bound with brain membrane receptors [2, 6, 11] or on artificial membranes [10], can itself be bound with antitoxin. The present writers [3, 12, 13] have put forward the idea that low-molecular-weight fragments of antitoxin, possessing specific activity, and with a reduced size of their molecule, might be more effective if they passed more easily through the blood–brain barrier and penetrated into the brain. Investigations [5] have shown that such antitoxin fragments (Fab'-fragments) neutralize more effectively than the ordinary Soviet "Diaferm-3" antitoxin, tetanus toxin bound to protagon, which is a complex of gangliosides with cerebrosides isolated from brain and specifically binding tetanus toxin [15].

The aim of the present investigation was to study the effectiveness of antitoxin with different molecular weight on various stages of tetanus intoxication in laboratory animals of different species.

EXPERIMENTAL METHOD

Pseudoglobulin, extracted with ammonium sulfate from high-affinity horse antitetanus serum, prepared at Stavropol' Research Institute of Vaccines and Sera, was subjected to proteolysis by trypsin, chymotrypsin, papain, and pronase. Better results were obtained by the use of crystalline "Difco" trypsin with an enzyme–substrate ratio of 1:50; pH 8.5; 37°C, for 24 h in the presence of 0.02M cysteine. The coagulated proteins were removed by centrifugation and the supernatant was fractionated on Sephadex G-100 (column 35 × 3.5 cm) in 0.15M NaCl solution, and the fractions collected on an XKOB-1 automatic collector, identified in the agar gel precipitation inhibition reaction between concentrated tetanus toxoid and "Diaferm-3" antitetanus serum, pooled, and concentrated by dehydration against polyethylene-

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TABLE 1. Effect of Type and Doses of Antitoxins on Survival of Mice with Tetanus Intoxication Caused by Intravenous Injection of Tetanus Toxin (M ± m)

Dose of toxin, DCL	Number of animals	Antitoxin		Did not develop disease	Developed disease and died	Length of survival, days
		type	dose, IU/kg			
1	20	Control	—	0	20	3,6±0,7
2	15	»	—	0	15	2,5±0,5
1	25	Diaferm-3	1 000	9	16	—
1	20	»	10 000	13	7	—
1	20	»	20 000	14	6	—
2	15	»	1 000	0	15	4,3±0,6
2	15	»	10 000	0	15	4,0±0,8
2	15	»	20 000	0	15	3,7±0,7
1	25	Fab'-fragment	1 000	10	15	—
1	20	»	10 000	14	6	—
1	20	»	20 000	18	2	—
2	15	»	1 000	0	15	3,7±0,7
2	15	»	10 000	0	15	4,0±1,0
2	15	»	20 000	0	15	4,3±1,2

TABLE 2. Effectiveness of Injection of Various Antitoxins by Different Method into Rats with Tetanus Intoxication Caused by Intravenous Injection of Tetanus Toxin (M ± m)

Antitoxin				Animals		Length of survival, days
type	dose, IU	route of injection	interval, h	total number	number which survived	
toxin control	—	—	—	36/0		3,8±0,6
Diaferm-3	1500	i/c	24	8/5		5,3±2,0
»	1500	i/v	24	8/1		5,0±1,8
»	1500	i/c	48	11/0		4,4±1,3
»	1500	i/v	48	8/0		4,2±1,2
»	3000	i/c	72	11/0		4,0±1,0
»	3000	i/v	72	10/0		3,9±0,7
Fab'-fragment	1500	i/c	24	9/7		5,5±0,7
»	1500	i/v	24	10/5		5,4±1,4
»	1500	i/c	48	13/0		4,9±1,2
»	1500	i/v	48	10/0		4,4±0,8
»	3000	i/c	72	19/0		4,1±1,1
»	3000	i/v	72	11/0		4,0±0,9

glycol (40 kilodaltons) in the cold to 10% protein. The molecular weight of the Fab'-fragments, as shown by gel-filtration, thin-layer chromatography, gel-diffusion, and viscosimetry, was more than 45 kilodaltons, with specific activity of 10-12 IU/mg protein. Activity of the Fab'-fragments was equal to that of "Diaferm-3" antitoxin. Rabbits weighing 2-3 kg, albino rats weighing 180-200 g, and albino mice weighing 18-20 g were used in the experiments. Tetanus was induced by injection of 1 DCL of tetanus toxin for animals of each species.

Effectiveness of the antitoxin injected in different ways was judged from lengthening of survival of the animals or their recovery. Antitoxin was injected intravenously, into the carotid artery, or intrathecally at different time intervals after the toxin, i.e., at different stages of intoxication.

Animals which could not take food unaided because of trismus were fed parenterally with a nutrient mixture consisting of 20 ml protein hydrolysate of "Aminokrovin" type, 20 ml of medium No. 199, and 1 g glucose per kilogram body weight. Streptomycin was given to all animals in a daily dose of 25,000 U/kg to prevent microbial complications.

EXPERIMENTAL RESULTS

In the experiments of series I, conducted on 63 rabbits, the animals were given 1 DCL* of tetanus toxin intravenously or into the carotid artery, followed after different time in-

*Conventional lethal dose.

TABLE 3. Effectiveness of Antitoxins Injected by Different Routes

Type of intoxication	Antitoxin: route of injection	Antitoxin: type	Number of animals	Length of survival, days: 1–2	3–5	6–17	Number which survived
	Control		13	—	13	—	0
Ascending	Interval, h	Diaferm-3	9	0	7	2	0
		Fab'-fragment	11	1	8	2	0
	Animals	Diaferm-3	16	0	4	5	7
		Fab'-fragment	8	5	2	1	0
	Control		14	0	14	0	0
Generalized	Interval, h	Diaferm-3	13	1	7	3	2
		Fab'-fragment	39	2	31	5	1
	Animals	Diaferm-3	19	0	5	12	2
		Fab'-fragment	9	4	3	2	0
	Control		10	0	10	0	0
Cerebral	Interval, h	Diaferm-3	7	5	0	1	1
		Fab'-fragment	8	5	2	1	0
	Animals	Diaferm-3	16	4	2	3	7
		Fab'-fragment	8	5	2	0	1

tervals by similar or crossed injection of "Diaferm-3" antitoxin or its Fab'-fragments in a dose of 1000 IU/kg.

The investigations showed that "Diaferm-3" antitoxin, injected into the blood stream, had a neutralizing action only until 4 h after injection of the toxin, and after 5 h it no longer prevented the development of toxic manifestations, whereas the Fab'-fragments neutralized the toxin for 7-8 h.

In the experiments of series II the protective effect of intravenous injection of antitoxin 18-20 h after intravenous injection of the toxin was studied in mice.

It can be concluded from Table 1 that Fab'-fragments on antitoxin had a somewhat better protective effect than "Diaferm-3" in animals receiving an injection of 1 DCL of toxin. When the dose of toxin was increased to 2 DCL the antitoxin (both Fab'-fragments and "Diaferm-3"), even in doses of 20,000 IU/kg, injected intravenously 18-20 h after the toxin, proved ineffective.

Table 2 gives the results of a study of the therapeutic efficacy of antitoxin in the treatment of rats with tetanus intoxication caused by intravenous (i/v) or intracarotid (i/c) injection of toxin.

As Table 2 shows, Fab'-fragments of antitoxin, injected by the intracarotid route in the early stages of intoxication (24 h) were somewhat more effective than "Diaferm-3". With lengthening of the interval between injection of toxin and antitoxin to 48-72 h no difference in efficacy could be observed.

In the experiments of series IV on rabbits with ascending (injection of toxin into muscles of the hind limb), generalized (injection of toxin into the blood stream), and cerebral (injection of toxin into the CSF) forms of intoxication, the therapeutic efficacy of antitoxin was studied when injected by different routes in the early stages of already developed intoxication (24-48 h).

On the appearance of signs of intoxication in the animals (usually after 24 or 48 h depending on the type of intoxication), "Diaferm-3" antitoxin or Fab'-fragments were injected by intracarotid or intracisternal routes and the development and outcome of the intoxication were observed. The dose of the antitoxins was 1000 IU/kg for intracarotid and 400 IU/kg for intracisternal injections. The results are given in Table 3.

Comparison of the therapeutic efficacy of "Diaferm-3" antitoxin with its Fab'-fragments showed that the latter had no advantage. Only two of the 83 rabbits treated with Fab'-fragments survived (2.4%) these experiments whereas, of 80 treated with "Diaferm-3" antitoxin, 19 (23.8%) survived.

Comparison of the results of treatment depending on the route of injection of the antitoxins showed that intracisternal injection had a considerable advantage over intracarotid

in all three types of intoxication: of 83 animals into which antitoxin was injected by the intracarotid route only four (4.8%) survived, whereas of 80 treated by intracisternal injection of antitoxin 17 (21.3%) survived.

The results of these experiments show that Fab'-fragments of antitoxin are rather more effective than "Diaferm-3" in the early stages of intoxication, if very large doses are injected and, in particular, by the intracarotid route. This result can be explained by the relatively greater penetration of Fab'-fragments into brain tissue [9, 16]. However, the differences observed are small and disappear with the development of intoxication. In the late stages of the process and in intoxication caused by increased doses of toxin, monovalent Fab'-fragments are just as ineffective as the "Diaferm-3" bipolar antitoxin, due to the more rapid excretion of Fab'-fragments from the body [1, 4, 8].

Intracisternal injection of "Diaferm-3" antitoxin proved to be the most effective method of treatment of already developed intoxication, as the writers showed previously in animals of other species [7].

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