COMPETITION BETWEEN TETANUS TOXOID AND TOXIN

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Experiments on albino mice showed that preliminary injection of tetanus toxoid increases the resistance of animals to tetanus toxin, as manifested by an increase in LD_{50}. The effect is enhanced by increasing the dose of toxoid or by giving it in fractional doses. The use of protagon and unpurified mitochondrial fraction, isolated from the brain, as receptor of tetanus toxin in the nerve tissue revealed competition for substrate between the tetanus toxoid and toxin. The results of these experiments confirm the writers' earlier hypothesis that the tetanus toxin molecule contains different functional groups responsible for binding the toxin with the receptor in brain tissue, for the pathogenic action of the toxin, and for the binding of the toxin with antitoxin.

KEY WORDS: tetanus toxin; toxoid; phenomenon of early protective action of toxoid; receptor of tetanus toxin; functional groups in toxin molecule.

The writers previously [4, 5, 10] postulated that the tetanus toxin molecule possesses three functional groups: an antigenic group responsible for binding with antitoxin, a toxophore group responsible for the pathogenic effect, and a group responsible for binding the toxin to the brain or spinal cord receptor (gangliosides). The conclusion that the toxophore group is unnecessary for binding of the toxin follows from data [16] showing that toxoid, which has no toxic properties, can be bound by the nerve-tissue receptor. This fact, however, required special examination. It can be considered in conjunction with the well-known [1, 2, 6, 12, 13, 18, 19, 22, 23] phenomenon of the early protective action of toxoid, in which, if suitable doses of toxoid are first injected into an animal, the action of toxin is subsequently weakened. This phenomenon was provisionally explained by the presence of competition between the toxin and toxoid. This hypothesis required direct experimental proof. The investigation described below was carried out to study these problems.

EXPERIMENTAL METHOD

Solutions containing 5-100 fixation units (f.u.) of concentrated tetanus toxoid, batch No. 56, and 0.0006-0.4 μg dried or purified (by Pillemer's [21] method) tetanus toxin, batch No. 228 (Leningrad Research Institute of Vaccines and Sera), were injected into the muscles of the right and left hind limbs of albino mice. The severity of tetanus poisoning was measured in LD_{50} units by Kerber's statistical method in the modification of Ashmarin and Vorob'e [3]. For the determination of LD_{50}, in each series of experiments four successively increasing doses of toxin were used, and each dose of toxin, with the corresponding effect of toxoid, was tested on 20 mice. In each series of experiments 80 mice thus were used. The same number of mice were used to determine LD_{50} in the control.

Purified tetanospasmin, labeled with I^{131}, was obtained by the method adopted previously [7, 8, 9]. Sarcosomes from the thigh muscles of guinea pigs were obtained as described by Chappel and Perry [11], the unpurified mitochondrial fraction (UMF) was obtained from guinea pigs' brains by the method of Schneider

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TABLE 1. Effect of Toxoid on Pathogenic Effect of Toxin (from LD_{50} values of toxin)

Experimental conditions	Dose of toxoid (in f.u.)	Doses of toxin (in μg)	No. of mice		LD_{50} (in μg)	Changes in LD_{50} (number of times)
			total	died		
Control	—	0,005	20	20		
		0,0025	20	16		
		0,0012	20	4	0,0017	
		0,0006	20	0		
Single injection of toxoid						
8 h before toxin	10	0,005	20	20		
		0,0025	20	10		
		0,0012	20	6	0,002	1,17
		0,0006	20	0		
4 h before toxin	10	0,005	20	20		
		0,0025	20	8		
		0,0012	20	6	0,0021	1,2
		0,0006	20	0		
2 h before toxin	10	0,005	20	20		
		0,0025	20	12		
		0,0012	20	4	0,002	1,17
		0,0006	20	0		
simultaneously with toxin	10	0,005	20	20		
		0,0025	20	16		
		0,0012	20	2	0,0019	1,1
		0,0006	20	0		
Fractional injections of toxoid						
8, 6, 4, 2 h before toxin and simultaneously with it	10 (5 times, 2 each time)	0,04	20	20		
		0,02	20	20		
		0,01	20	16	0,007	4,1
		0,005	20	6		
4, 3, 2, 1 h before toxin and simultaneously with it	10 (5 times, 2 each time)	0,04	20	20		
		0,02	20	18		
		0,01	20	16	0,0073	4,3
		0,005	20	4		
60, 45, 30, and 15 min before toxin and simultaneously with it	10 (5 times, 2 each time)	0,04	20	20		
		0,02	20	20		
		0,01	20	13	0,0075	4,5
		0,05	20	2		
60, 45, 30, and 15 min before toxin and simultaneously with it	5 (5 times, 1 each time)	0,02	16	16		
		0,01	16	14		
		0,005	16	10	0,0046	2,7
		0,0025	16	2		
60, 45, 30, and 15 min before toxin and simultaneously with it	20 (5 times, 4 each time)	0,04	16	16		
		0,02	16	15		
		0,01	16	9	0,0094	5,5
		0,005	16	3		
60, 45, 30, and 15 min before toxin and simultaneously with it	100 (5 times, 20 each time)	0,08	16	16		
		0,04	16	14		
		0,02	16	13	0,012	7,0
		0,01	16	6		

TABLE 2. Effect of Tetanus Toxoid on Fixation of Labeled Toxin by Substrates (M ± m)

Experimental conditions	Quantity of toxin-I^{131} fixed (in μg/mg substrate)		
	brain UMF	protagon	muscle sarcosomes
Simultaneous incubation with toxoid			
Substrate + toxin (control)	0,35±0,02	0,45±0,02	0,23±0,16
Substrate + toxin + 10 f. u. toxoid	0,30±0,017 $P>0,01$	0,305±0,015 $P>0,01$	0,20±0,01 $P>0,01$
Substrate + toxin + 100 f. u. toxoid	0,175±0,01 $P<0,005$	0,184±0,009 $P<0,005$	0,17±0,014 $P>0,01$
Preincubation with toxoid			
Substrate + toxin (control)	0,32±0,019	0,38±0,017	0,22±0,016
Substrate + 10 f. u. toxoid + toxin	0,27±0,018 $P>0,01$	0,25±0,012 $P>0,01$	0,175±0,017 $P>0,01$
Substrate + 100 f. u. toxoid + toxin	0,15±0,01 $P<0,005$	0,152±0,006 $P<0,005$	0,14±0,009 $P<0,005$

Legend. Each value is the mean of 9-11 determinations.

and Hogeboom [24], and protagon, an unpurified complex of cerebrosides and gangliosides, used as the toxin receptor [17] was obtained from bovine brain by the method of Wilson and Gramer [25] in Promyslov's modification.

Freshly prepared toxin-I^{131} was added to the substrates at the rate of 1 μg to 1 mg sarcosomes from the muscles and brain UMF or to 1 mg protagon. Tetanus toxoid was added in doses of 10 and 100 f.u. per sample.

In the experiments of series I, toxin-I^{131} and toxoid were incubated simultaneously with protagon in physiological saline for 45 min, but with brain UMF and muscle sarcosomes they were incubated in Gubler's medium [14] for 10 min at 37°C. The mixtures were precipitated and washed twice by centrifugation at 12,000 rpm, for 15 min each time, to remove traces of unbound toxin-I^{131}.

In the experiments of series II, protagon, sarcosomes, or UMF were preincubated under the same conditions with the same doses of toxoid (without toxoid in the control), after which they were washed twice by centrifugation, and the protagon was then incubated for 45 min and the sarcosomes and UMF for 10 min each with toxin-I^{131} at 37°C. The test fractions were again sedimented and washed twice by centrifugation. Protein was estimated in the preparations by Lowry's method [20].

EXPERIMENTAL RESULTS

Preliminary injection of a single dose of 10 f.u. toxoid into mice was shown to increase LD_{50} for tetanus toxin by only 15%, whereas the same dose of toxoid, if divided into five injections, increased LD_{50} by more than five times (Table 1). The effectiveness of the 10 f.u. toxoid was somewhat greater if the fractional injections were given 60 min than if given 8 h before injection of the toxin. The same tests showed that the rapid protective action of toxoid against tetanus poisoning increases only with an increase in the dose of toxoid to 100 f.u.

The possibility of competition between toxoid and toxin for the receptor was studied in experiments in vitro with protagon, brain UMF, and muscle sarcosomes from guinea pigs. The results showed that simultaneous incubation of the substrates with toxoid and toxin-I^{131} and preliminary saturation of the substrates with toxoid reduced fixation of the toxin by the receptor. Binding of toxin-I^{131} by UMF was reduced by 50% in the case of simultaneous incubation with 100 f.u. toxoid and by 53% after preliminary treatment of the UMF with the same dose of toxoid (Table 2). Fixation of labeled tetanospasmin by protagon during simultaneous incubation with toxoid was reduced by 59% and after preliminary incubation of toxoid with protagon by 60%. No such marked effect was observed in the experiments with muscle sarcosomes. The action of tetanus toxoid in a dose of 100 f.u. reduced fixation of tetanus toxin by the sarcosomes by 16.1-36.4%.

After both simultaneous and preliminary incubation of protagon and brain UMF with the toxoid, a competitive effect of the toxoid was thus observed on fixation of the toxin by substrates containing the toxin receptor. This fact agrees with observations showing that after treatment with toxoid, brain tissue homogenate binds less toxoid [15].

The results of these experiments, indicating competition between toxoid and toxin for the receptor, can explain the phenomenon of early protective action of toxoid. They support the earlier hypothesis that tetanus toxin is not bound with the receptor by the toxophore group, for toxoid, which has no toxic properties, also binds with the receptor and that the toxin molecule contains at least three functional groups; one of these groups – the receptor-binding group – is present in both toxin and toxoid and is responsible for binding toxin (and toxoid) with the nerve-tissue receptor [4, 5, 10].

In conclusion, competitive relations between toxin and toxoid may occur not only in the central nervous system but also at the periphery, for example, at the portal of entry into the neural pathway whereby the toxin reaches the CNS and, possibly, in the neural pathway itself.

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