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Short Communication

USE OF MONOCLONAL ANTIBODIES IN ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)
FOR DETECTION OF BOTULINUM TYPE B TOXINS

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SUMMARY: Use of polyclonal antibodies failed to correlate mouse assay with enzyme linked immunosorbent assay (ELISA) in titration of culture fluid of different strains of *Clostridium botulinum* type B. If ELISA is performed with such a monoclonal antibody that is capable of neutralizing the toxin, however, the lethal toxicity can be determined quantitatively.

Clostridium botulinum type B includes proteolytic and nonproteolytic strains. The toxins produced by these strains are considered to be heterogenous (1,2). Type B progenitor toxin is composed of two components, toxic and nontoxic (3). The toxic component or derivative toxin (about 170,000 daltons) is made up of two fragments of about 110,000 daltons (fragment I) and 59,000 daltons (fragment II). The immunological difference found between the toxins produced by proteolytic and nonproteolytic strains of *C. botulinum* type B was ascribed to the antigenic difference of fragment I (4). Miyazaki et al. (5) found different potential toxicities between the two type B toxins.

Such methods for in vitro assay of botulinum toxin as radioimmuno assay (RIA) and enzyme linked immunosorbent assay (ELISA) are based on the immunological reaction between the toxin and its antibodies. Due to the immunological and the biological heterogeneity, it may be anticipated that, in testing the toxins produced by proteolytic and nonproteolytic type B strains, the results of ELISA will differ from those of bioassay. In this investigation, ELISA using polyclonal and monoclonal antibodies was compared with the mouse test for detecting type B toxins produced by *C. botulinum* type B strains okra, B7273, CDI III, SNB77 (proteolytic), CDI I and CDI II (nonproteolytic). Each strain was transferred to fortified egg meat medium (6) and the cultures were incubated anaerobically at 30 C for 5 days.

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Culture fluids were titrated by ELISA and the mouse test. Progenitor toxins (both B-L and B-M toxins) of strain okra were purified according to Kozaki et al. (3). The toxic component (derivative toxin) was isolated from B-M toxin by DEAE-Sephadex chromatography at pH 8.0.

Polyclonal antibodies (PCA) used for ELISA was an IgG fraction of the sheep immunized with the derivative toxin. Monoclonal antibodies (MCA) were obtained by hyperimmunizing mice with the formalinized derivative toxin. The spleen cells of the mouse were mixed with the myeloma cells; the hybridoma cells obtained were tested for production of anti-derivative toxin. Four cell lines (B-4-1, B-4-2, B-6-2 and B-6-4) were obtained. The antibodies were obtained by injecting the hybridoma cells into mice. MCA B-6-4 alone neutralized type B toxin.

Horse radish peroxidase (Sigma type VI) was conjugated to PCA by the method of Nakane and Kawaoi (7). Sandwich ELISA was utilized as described by Notermans, Dufrenne and Kozaki (8). For coating, either PCA or each MCA was used. As conjugate, PCA was used. The peroxidase activity was determined with 5-aminosalicylic acid as chromogenic substance.

Toxin potencies were determined after tryptic activation. Serial twofold dilutions were made and 0.5-ml doses of each dilution were injected intraperitoneally into four mice. LD₅₀ was calculated in 4 days according to Reed and Muench (9).

The relations between ELISA and bioassay for detecting type B toxins produced by different strains are presented in Table I. With PCA, the minimum detectable

Table I. *Relation between ELISA and mouse assay for detection of type B botulinum toxin*

Toxin	log mouse ip LD ₅₀ /ml (after tryptic treat- ment)	Minimal detectable quantity of toxin in log mouse ip LD ₅₀ /ml by ELISA with coating antibody				
		PCA	B-4-1	B-4-2	B-6-2	B-6-4
Okra (P*)	6.68	0.9	4.1	> 5.6	2.7	2.6
B7273 (P)	6.45	1.1	4.0	> 5.5	2.6	2.6
SNB77 (P)	5.32	1.9	> 5.0	> 5.0	2.6	2.6
CDI III (P)	5.41	1.8	> 5.1	> 5.1	2.5	2.5
CDI I (NP**)	5.86	2.9	> 5.2	> 5.2	2.9	2.9
CDI II (NP)	5.71	2.8	> 5.2	> 5.2	2.9	2.8
B-M	--	2.0	3.8	4.8	2.9	2.8
B-L	--	2.1	3.9	4.9	2.8	2.9

* Toxin of a proteolytic strain.

** Toxin of a nonproteolytic strain.

quantity of toxin (expressed in mouse ip LD₅₀) by ELISA varied depending on the strain tested. The minimal detectable amount of okra toxin was about 10 mouse ip LD₅₀/ml, while that of CDI I toxin 700-800 mouse ip LD₅₀/ml. When MCA B-6-2 or B-6-4 was used for coating, however, the minimal detectable amounts were the same for all strains tested, being 300-800 mouse ip LD₅₀/ml. These results suggest that both MCAs react with a common antigenic site of type B toxin produced by proteolytic and nonproteolytic strains.

With MCA B-4-1 as coating antibody, only the toxins produced by proteolytic strains okra and B7273 were detectable. With MCA B-4-2, however, none of the toxins produced by the strains tested was detectable, whereas purified B-L and B-M toxins were detected. All the experiments were performed with culture fluid and purified toxin diluted in 0.07 M PBS, pH 7.2 without any further treatment.

When culture fluid was treated with trypsin, positive reaction occurred with culture fluids of strains okra, B7273, CDI III and SNB 77 with MCA B-4-2 as coat. With MCA B-4-1 as coat, positive reaction occurred with trypsin-treated culture fluids of strains CDI III and SNB 77. Since positive reactions were observed only with 1:50 or lower dilutions of culture fluids, it can not be ruled out that the reactions were nonspecific. Tryptic treatment of B-L and B-M toxins gave minimal detectable amounts of about 10⁶ mouse ip LD₅₀/ml or larger with MCA B-4-2 as coat.

When the toxins were diluted in 0.07 M PBS, pH 8.3, no beneficial effect was furnished, but the minimal detectable amount of both B-L and B-M toxin became approximately 10⁴ mouse ip LD₅₀/ml with MCA B-4-2 as coat. This finding may indicate that MCA B-4-2 reacts with an antigenic site situated close to the site binding to the nontoxic component.

From these results, it seems that if carefully selected MCAs are used, the same amounts of biologically active type B toxins can be determined by ELISA regardless of the origin of the toxin. There may be at least two sites determining the toxicity of botulinum toxin, the site binding to the nerve ending (synaptosome) and the site inhibiting acetylcholine release (10). Kozaki (11) demonstrated that the site binding to the synaptosome is situated on fragment I of the derivative toxin. It seems then possible that the site inhibiting acetylcholine release is located on fragment II. There has been no clear evidence, however, to support this hypothesis. Determination of only the biologically active part of the derivative toxin will favor the in vitro assay. Sandwich ELISA may be fitted for this purpose, since as coating antibody MCA reacting with the binding site of the toxin and as enzyme conjugate MCA reacting with the toxic site of the molecule can be used.

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