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Botulinum type A toxin neutralisation by specific IgG and its fragments: A comparison of mouse systemic toxicity and local flaccid paralysis assays

R.G.A. Jones[a,*], T.-A. Alsop[a], R. Hull[b], R. Tierney[a], P. Rigsby[c], J. Holley[d], D. Sesardic[a]

[a]*Division of Bacteriology, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire. EN6 3QG, UK*
[b]*BSS, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire. EN6 3QG, UK*
[c]*Informatics, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire. EN6 3QG, UK*
[d]*Dstl, Porton Down, Salisbury, Wiltshire, SP4 0JQ, UK*

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Abstract

In this study, we have compared two in vivo assay methods to measure the type A botulinum toxin neutralising activity of specific immunoglobulin G (IgG) and its fragments ($F(ab')_2$, Fab′, Fab) purified from pentavalent botulinum antisera raised in goats. Each assay method was repeated on three separate occasions in mice and relative potencies calculated with respect to a type A equine reference antitoxin. The conventional assay, which measures the number of mice surviving typically after 72 or 96 h following the intraperitoneal administration of a mixture of toxin and antitoxin, gave the following order of potency IgG > $F(ab')_2$ > Fab′ > Fab (6.8 > 4.7 > 3.5 > 2.6 IU/mg). Differences in potency are likely to be due to differences in the pharmacokinetics of the antitoxins, which are related to their molecular weight. The alternative local flaccid paralysis assay, where toxin and antitoxin are injected subcutaneously into the left inguinocrural region, gave results with a narrower range of activities: IgG > Fab′ > $F(ab')_2$ > Fab (6.0 > 5.9 > 5.5 > 4.6 IU/mg). Comparison of the two assay methods showed no significant differences for IgG, $F(ab')_2$ or Fab′, although the Fab fragment was significantly more potent in the non-lethal assay probably because of the reduced influence of antitoxin pharmacokinetics in this localised assay. These findings show that a local flaccid paralysis assay provides a less time consuming and more humane alternative to the lethal assay for the potency testing of botulinum IgG and $F(ab')_2$ antitoxins.

Keywords: Botulism; Antitoxin; In vivo; Immunoglobulin; Toxin

1. Introduction

Seven types or serotypes (A, B, C, D, E, F and G) of botulinum toxin are currently known. Each serotype is composed of a heavy and a light chain linked together by disulphide bonds. The N-terminal end of the heavy chain is responsible for binding to specific pre-synaptic neuronal cell receptors (Synaptic Vesicle protein 2 and gangliosides, for type A toxin) and the C-terminal end facilitates internalisation (Montecucco et al., 1994;

*Corresponding author. Fax: +44 01707 663796.
E-mail address: rjones@nibsc.ac.uk (R.G.A. Jones).

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Dong et al., 2006). Once inside the cell, the light chain transverses the membrane of the endocytolic vesicles and cleaves specific proteins essential for the fusion of neurotransmitter containing vesicles (Schiavo et al., 1992, 2000). Subsequently, the extracellular release of neurotransmitter into the neuromuscular junction is blocked and results in a flaccid muscular paralysis which can ultimately lead to death.

A dose of >50 ng of botulinum toxin per person typically causes a life-threatening, paralysing disease called botulism. Although rare, botulism can be caused by the ingestion of food contaminated with either botulinum spores (infant or intestinal botulism) or contaminated with toxin (food-borne botulism). Another form of botulism is caused by spore contamination of traumatised tissue (wound botulism). More recently, the threat of botulinum toxin used as a biological weapon, which would cause inhalational botulism, has been identified. Naturally occurring botulism in humans, including infant botulism, is generally associated with serotypes A, B and E toxins. Specific treatment is available in the form of intravenously or intramuscularly administered antitoxin (Shapiro et al., 1998; Mayers et al., 2001; Hibbs et al., 1996). Specific antitoxins typically consist of hyperimmune equine $F(ab')_2$ preparations containing not less than 500 IU/ml of each of types A and B, and 50 IU/ml of type E antitoxin (PhEur monograph, 2006a). Investigational heptavalent preparations have also been developed against A–G toxins (Hibbs et al., 1996), and human-derived botulinum antitoxin (BIG) purified from pentavalent toxoid (A, B, C, D, E) vaccinated individuals, with $\geqslant 15$ IU/50 mg immunoglobulin for type A antitoxin, has been successfully used in an infant botulism trial in California (Hibbs et al., 1996; Sesardic et al., 2000; Arnon, 1993). Although the availability of BIG is limited, it has recently gained regulatory (FDA) approval (Arnon et al., 2006).

The safety of antitoxins and antivenoms is known to vary between products manufactured in different ways, due to the purity, immunogenicity, potency and dose (Chippaux et al., 1999; Smith et al., 1979; Mayers et al., 2001; Bleeker et al., 1987). Typically, a 9% incidence of side effects is observed with botulinum and other $F(ab')_2$ products (Black and Gunn, 1980; Chippaux et al., 1999); however, more purified products have a greatly reduced incidence of side effects ranging from $<1\%$ or 4% with highly purified ovine Fab and equine $F(ab')_2$ products, respectively (Kirkpatrick, 1991; Chippaux et al., 1999). These improved products are considered safe, typically with only mild adverse effects; however, by contrast, much higher incidences of side effects from 22% to 76% are seen with poorly processed equine $F(ab')_2$ products (Hibbs et al., 1996; Moran et al., 1998).

With recent world events, the need for antitoxins to combat the bioweapons threat has been highlighted especially with the absence of a licensed vaccine suitable for prophylactic use. The increasingly widespread clinical use of therapeutic botulinum toxins (A and B) and possible future use of re-engineered toxin components or different serotypes, however, may make the vaccination of the general public ethically unacceptable even if a vaccine was available. There is currently, therefore, a demand for a new generation of safe and highly potent botulinum antitoxins against all seven toxin serotypes, suitable for treating the general public and emergency services following a release incident. New ovine and equine $F(ab')_2$ products are now being manufactured to bridge this gap.

The mouse lethality bioassay is currently the most reliable procedure to estimate the potency of botulinum antitoxins (PhEur monograph, 2006a). This approach uses large numbers of laboratory animals and is time consuming and expensive. A substantial amount of effort has been spent finding alternative methods to try to replace the mouse lethality (LD_{50}) bioassay (Szilagyi et al., 2000; Ferreira, 2001; Welch et al., 2000; Ekong et al., 1997). For instance, a non-lethal in vivo muscular paralysis assay and an in vitro endopeptidase assay have been developed and validated for measuring the potency of therapeutic BoNT/A for batch release purposes to check that toxin levels fall between the stated limits (Sesardic et al., 1996; PhEur monograph, 2006b). A combination of these assays has provided a real alternative with the subsequent refinement and replacement in the use of the lethal assay procedure.

Although the endopeptidase assay measures the enzymic activity of botulinum toxin, it is not ideal for measuring antitoxin potency since antibodies recognising critical regions of the H-chain, which would inhibit uptake into neurones and subsequent toxicity, are unlikely to be detected. Reliable alternatives, which measure the full biological activity of the toxin and antitoxin, are required and essential for testing of new generation products.

The following study was performed to compare the conventional systemic lethal assay and a