Experience with the Use of an Investigational F(ab')2 Heptavalent Botulism Immune Globulin of Equine Origin During an Outbreak of Type E Botulism in Egypt


During an outbreak of type E foodborne botulism in Cairo in 1991, an investigational equine F(ab')2, "despeciated" heptavalent botulism immune globulin (dBIG) was provided to the Egyptian Ministry of Health by the U.S. Army. Of 54 patients known to have been treated with antitoxins, 4 received commercially available trivalent antitoxins, 45 received dBIG, and 5 received both commercial antitoxin and dBIG. Physicians recorded side effects in 10 (22%) of 45 patients who received dBIG; in nine cases, reactions were considered "mild," and in one case they were believed to be serum sickness. In contrast, possible serum sickness during hospitalization was recorded for two of four patients who were receiving commercial antitoxins. No complications of therapy were noted for any patient who was receiving both antitoxin types. In a separate study, 31 patients were contacted about their reactions to the antitoxin by telephone after discharge from the hospital. Seven (54%) of 13 patients attributed symptoms that they experienced while they were hospitalized to receipt of dBIG, while four (44%) of nine patients who indicated that they had received commercial antitoxins and one (20%) of five who received both commercial antitoxin and dBIG reported side effects before discharge. Data on the efficacy of the antitoxins were not obtained. In our experience, equine dBIG was at least as safe as commercially available antitoxins in treating type E foodborne botulism.

Botulism is a paralytic illness that occurs when neuromuscular transmission is interrupted by a protein neurotoxin elaborated by the spore-forming, obligate anaerobic bacterium Clostridium botulinum. At least seven distinct toxin types have been identified, five of which (A, B, E, F, and G) have been associated with human botulism. Foodborne botulism occurs after ingestion of preformed toxins in foods contaminated with C. botulinum; type E botulism most commonly is associated with consumption of fish or fish products.

Standard therapy for foodborne botulism includes intensive supportive care and administration of botulinal antitoxin [1]. An equine trivalent (types A, B, and E) product manufactured by Connaught Laboratories (Willowdale, Ontario, Canada) is the only botulinal antitoxin currently approved for use in the United States. However, a variety of other botulinal antitoxins, predominantly of equine origin, are manufactured for use outside this country.

For ethical reasons, no controlled trial to document the efficacy of botulinal antitoxin has been conducted in humans. However, reports suggest that patients with type A and type E botulism who have received trivalent equine antitoxin early in the course of illness fare better than those who have not [2-5].

Administration of commercially available equine antitoxin is complicated by its high cost (approximately $800 per two-vial dose) and the substantial risk of hypersensitivity reaction (9%) to whole horse-plasma globulin products or pepsin. Serum sickness develops in ~4% of individuals who receive equine antitoxin and increases with the number of vials received [6].

Recently, an extensively purified equine F(ab')2, "despeciated" heptavalent (against toxin types A, B, C1, D, E, F, and G) botulism immune globulin (dBIG) was prepared under contract for the U.S. Army [7]. Herein we summarize our experience with the use of dBIG during a massive outbreak of type E foodborne botulism that occurred in Cairo in April 1991 [8].

Methods

Details of the preparation and testing of dBIG are contained in the U.S. Food and Drug Administration's Investigational...
New Drug Application #3703. In brief, investigators at the University of Minnesota, under contract with the U.S. Army, prepared an equine antitoxin capable of neutralizing types A, B, C1, D, E, F, and G botulinal toxins. This heptavalent antitoxin was prepared for human use through an extensive purification procedure, which included use of SiO2 to eliminate fibrinogen, plasminogen, and other proteins. The equine plasma pool was then "despeciated" by treatment with pepsin in order to remove the Fc fragment of the immunoglobulin molecules. The resultant preparation was filtered and subjected to anion exchange and column chromatography (QAE-A50 Sephadex, Pharmacia Biotech, Piscataway, NJ) to isolate the F(ab')2 fragments. Following multiple tests for purity and potency, the final product was bottled under sterile conditions and made available for human testing.

Subsequent to written entry by the Egyptian Ministry of Health, 100 vials of dBIG were provided to the Egyptian government for compassionate use under an existing U.S. Department of Defense investigational protocol. The antitoxin was shipped to the U.S. Navy Medical Research Unit No. 3 in Cairo and controlled by their personnel. Investigators from the U.S. Navy and the Centers for Disease Control and Prevention provided officials from the Ministry of Health with dBIG and with a packet prepared for each patient that contained instructions, copies of the protocol, consent forms, enrollment forms, and all necessary supplies. The Ministry of Health distributed all materials, insured adherence to the protocol, and supervised the administration of consent forms and collection of data.

Under the protocol, individuals were tested for hypersensitivity before the antitoxin was administered by intradermal injection of a 1:10 dilution of dBIG; in addition, individuals were observed for potential reactions. Desensitization procedures were articulated in the protocol for skin test reactors. Each dose of dBIG consisted of a 10-mL vial administered intravenously over a 20-minute period.

In response to this outbreak, trivalent botulinal antitoxins had been obtained from commercial sources within and outside Egypt by private physicians and patients' family members. The types of antitoxin used were recorded in patient records as heptavalent (i.e., dBIG), French/Pasteur, French/Canadian, German, or a combination thereof. For purposes of analysis, we have grouped all non-dBIG preparations as "commercial botulinal antitoxins."

An investigation of the outbreak was conducted to determine its source and scope and is described elsewhere [8]. The cases of 91 patients who were hospitalized with suspected botulism were reported to the Ministry of Health (not all cases were laboratory confirmed, and 18 patients died). Available records indicated that no fewer than 54 patients in this group (59%) received botulinal antitoxin of at least one type during their hospital stay. Enrollment records for these 54 patients were made available to investigators by the Ministry of Health.

Forty-five of the 91 hospitalized patients had been previously interviewed by investigators during the outbreak investigation; 31 of these were contacted by telephone 2.5-4.5 weeks after discharge from the hospital, and, if symptoms were present, they were contacted again 2 months later. Telephone interviews were conducted in Arabic by one person, who read a questionnaire form. Patients were asked such questions as whether they remembered receiving antitoxin, which symptoms they had had during hospitalization and on discharge, whether they had seen a doctor or had been readmitted, and whether they had had symptoms at 3 weeks and (as appropriate) at 3 months after discharge.

The differences between group proportions were assessed with use of Fisher's exact test. The differences between groups were defined as statistically significant at $P < .05$.

Results

Physician reports. Patient records and study enrollment forms from 54 patients hospitalized and treated with botulinal antitoxins were available for review by study investigators. Data were incomplete for 19 (35%) of these patients. However, from the information available, it could be determined that 50 of these patients received dBIG, either alone ($n = 45$) or in addition to a commercially available antitoxin ($n = 5$).

Ten (22%) of the 45 patients who received dBIG alone had side effects that were recorded by their physicians. Nine of these reactions were considered "mild"; these reactions included six local skin reactions (apparently following skin testing before administration of the antitoxin) and one instance each of pruritus, urticaria, and shivering (all considered a result of treatment). "Suggested serum sickness after 3 hours" was noted in one patient's record, but details were not provided. No adverse reactions attributable to therapy were recorded for any of the five patients who received commercial antitoxin plus dBIG. Two of the four individuals who received only commercial antitoxins sustained reactions charted only as "suspect serum sickness," but findings were not described further. There was no difference in frequency between reactions recorded for recipients of dBIG only and commercial antitoxin only ($P = NS$).

Patient interviews. A separate group of 45 patients had been interviewed by investigators while they were hospitalized with botulism; 31 of these discharged individuals (74% of the 42 who survived to discharge) who received at least one type of botulinal antitoxin could be contacted for telephone interview nearly 1 month (3.5 weeks) after discharge. Twenty-seven of the 31 persons could recall the type of antitoxin received.

Seven (54%) of 13 individuals who recalled receiving only dBIG reported symptoms that they attributed to antitoxin side effects (joint pain, rash, and/or flank pain) during hospitalization, while four (44%) of nine who recalled receiving only commercial antitoxin reported similar side effects (rash, pruritus, edema, hypotension, joint pain) during this period ($P = NS$). Of the five individuals who recalled receiving both dBIG and commercial antitoxin, only one reported side effects (rash
and pruritus). Joint pain was reported during hospitalization more frequently by recipients of dBIG only (46%) than by recipients of commercial antitoxin only (11%), but this difference was not statistically significant \( (P = \text{NS}) \)

Considering only those complaints that could be plausibly linked to receipt of antitoxin (joint pain, rash, edema, pruritus), 6 (46%) of 13 who received dBIG only, 4 (44%) of 9 who received commercial antitoxin only, and 1 (20%) of 5 who received both dBIG and commercial antitoxin sustained reactions possibly related to administration of antitoxin. Although no patient reported symptoms that met rigorous case definitions for serum sickness, no recipients of dBIG, two recipients of commercial antitoxin (22%), and one recipient of both antitoxins (20%) reported reactions that may have been consistent with a serum sickness–like syndrome [9–11].

Eight (30%) of the 27 patients contacted reported persistent complaints at the time of the first telephone interview. However, only one of these patients (who received commercial antitoxin only) reported symptoms (joint pain) potentially related to receipt of antitoxin. Of the eight persons reporting symptoms at 1 month after discharge, five were available for interview 3 months after their discharge. Three of five patients who had persistent complaints, none of whom were in the group who recalled receiving only dBIG. One person (a recipient of commercial antitoxin) reported residual joint pain (the only complaint that could be plausibly linked to receipt of antitoxin).

**Discussion**

Administration of botulinal antitoxin has been part of the routine management of botulism and suspected botulinal intoxications in humans since the 1960s [1]. An unacceptably high frequency of side effects, the need to administer skin tests to prospective recipients and to desensitize reactors, and the high cost of treatment courses have spurred efforts to develop alternatives to traditional equine antitoxins. One such product, a heptavalent F(ab')₂, botulinal immune globulin of equine origin, was developed by investigators under contract to the U.S. Army. This antitoxin, dBIG, proved effective in a mouse model for botulinal intoxication [12]. No adverse reactions were observed in a small study in which volunteers received a single 10-mL intravenous dose of dBIG [7]. The occurrence of a large foodborne outbreak of botulism in Egypt during which there was a governmental request for humanitarian assistance with dBIG afforded an opportunity to further evaluate its safety under field conditions.

The results of our assessment suggest that dBIG was at least as safe as commercially available antitoxins in the management of patients with type E foodborne botulism during this outbreak. Although conclusions cannot be drawn regarding the safety profile of this product in other forms of botulism, there is no biological basis for predicting a significantly different experience. Difficulties with recordkeeping, the fact that most fatalities occurred early in the epidemic when the diagnosis may have been unrecognized, the absence of laboratory confirmation of the diagnosis in many cases, and the inability to record other objective markers of product effectiveness unfortunately precluded any reasonable attempt to determine the efficacy of dBIG.

Although the numbers were small, data extracted from patient records and recorded by their physicians indicated that patients who received dBIG developed side effects related to therapy at a frequency similar to (and perhaps less than) those receiving commercially available antitoxins (22% vs. 50%). Data obtained by telephone interviews with patients nearly a month following hospital discharge supported these findings: 54% of dBIG recipients attributed symptoms that occurred during their hospitalization to the antitoxin while 44% of commercial antitoxin recipients made a similar association \( (P = \text{NS}) \).

Our experience with the use of dBIG was not unexpected. Most commercially available botulinal antitoxins are of equine origin. Hypersensitivity to antibody products of nonhuman origin generally are attributed to the extraneous proteins and Fc component. Pepsin removal of the Fc piece ("despeciation") and subsequent isolation and purification of the remaining F(ab')₂ fragments, as was done for dBIG, theoretically should reduce the potential for allergic responses upon reexposure to a sensitizing antigen such as horse protein.

There are clear shortcomings in both the quality and quantity of information available for analysis in our study. The number of patients who received commercially available antitoxins and from whom data were available was small. Data extracted from patient records were recorded by physicians who had limited experience in completing study forms and who were in the midst of a health care crisis. Patients' records frequently were incomplete, and full explanations of diagnostic conclusions often were not available. In some cases, clinicians were unfamiliar with botulism since this was the first recognized appearance of the disorder in Egypt.

Data obtained by telephone interviews was limited by the accuracy of recall and the relative degree of the patient's sophistication. Telephones in private residences are relatively scarce in Cairo; thus, individuals who were available for interview might not have been representative of all patients who received antitoxin during the outbreak. These patients could not provide detailed information on their hospital course, and there were no data on those who did not receive antitoxin. Some symptoms (e.g., joint pain) recalled by patients may have been related only to hospitalization; there was no "control" group of patients without botulism that could be compared with regard to these and other acute or residual complaints. Finally, information obtained from interviews was obviously biased to survivors. Data on antitoxins were available for only three fatal cases.

However, despite the flaws inherent in this study, the consistency of findings from our two information sources (i.e., physicians and telephone interviews) together with the absence of any serious adverse event report associated with use of dBIG.
support our conclusion that this "despeciated" heptavalent botulinal antitoxin was at least as safe as commercially available antitoxins under field conditions. It will be important to validate the safety of this product in future cases of human botulism and to carefully assess its effectiveness in treatment of this serious condition.

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References


