

Exposure-Response Modeling and Simulation to Support Human Dosing of Botulism Antitoxin Heptavalent Product

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Botulism antitoxin heptavalent (A, B, C, D, E, F, and G – Equine; BAT) product is a sterile solution of F(ab')₂ and F(ab')₂-related antibody fragments prepared from plasma obtained from horses that have been immunized with a specific serotype of botulinum toxoid and toxin. BAT product is indicated for the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin serotypes A to G in adults and pediatric patients. Pharmacokinetic and exposure-response models were used to explore the relationship between BAT product exposure and the probability of survival, and the occurrence of relevant moderate clinical signs observed during the preclinical development of BAT product to justify the clinical dose. The predicted probability of survival in humans for all serotypes of botulinum neurotoxin was more than 95.9% following intravenous administration of one vial of BAT product. Furthermore, this BAT product dose is expected to result in significant protection against clinical signs in human adults for all botulinum neurotoxin serotypes. Our exposure response model indicates that we have sufficient antitoxin levels to give full protection at various theoretical exposure levels and, based on neutralization capacity/potency of one dose of BAT product, it is expected to exceed the amount of circulating botulinum neurotoxin.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Botulinum neurotoxin is the most acutely lethal toxin known, but the traditional drug development paradigm for testing efficacious drugs such as botulism antitoxin heptavalent (A, B, C, D, E, F, and G – Equine; BAT) product against exposure in humans is unethical. A translational dose scaling model-based approach has been demonstrated to be a valuable tool for these various drugs approved under the “Animal Rule.”

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The objective of this research was to determine: (1) What types of quantitative approaches would support the translation of human dosing recommendations from animals? (2) Is the recommended dose of BAT product protective of the clinical population based on the available animal data?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This research suggests that translational dose scaling model-based approaches can be used successfully to support the translation of human dosing recommendations from animals. Using the available data to date and such a strategy, a BAT product vial with the current specification for neurotoxin neutralization is expected to result in significant protection against all clinical signs in humans for seven neurotoxin serotypes A to G.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ This research suggests that simple model-based approaches can help justify dose labelling decisions for drugs approved under the “Animal Rule.”

Botulinum neurotoxin is the most acutely lethal toxin known, with an estimated human median lethal dose (LD₅₀) of 1.3–2.1 nanograms per kilogram (ng/kg) intravenously (i.v.) or intramuscularly (i.m.) and 10 to 13 ng/kg when inhaled. A single gram of crystalline botulinum neurotoxin, evenly dispersed and inhaled, would be lethal to at least one million people.¹ The United States Centers for Disease Control and Prevention (CDC) has classified

botulinum neurotoxins as a category A biological warfare agent because of their extreme potency, ease of production, ability to be weaponized, and the resulting lethality or prolonged incapacity, leading to substantial disruption in societal functions.²

There are several antigenically distinct botulinum neurotoxins serotypes, designated by the letters A through G, and humans are susceptible to all of them.³ Recently, BoNT/X was identified as a

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unique branch of the botulinum neurotoxin family.⁴ The neurotoxin binds to peripheral cholinergic nerve terminals within the neuromuscular junction and is internalized via receptor-mediated endocytosis.⁵ Following neurotoxin internalization, the neurotoxin cleaves the protein complex involved in docking of the acetylcholine transport vesicle on the inner surface of the nerve cell membrane. This results in the inhibition of acetylcholine release. By blocking acetylcholine release, the neurotoxin causes the characteristic flaccid paralysis associated with botulism.⁶ Given the irreversible cleavage of these proteins in the presynaptic terminal, it is important for treatment to be introduced as soon as possible after exposure to botulinum neurotoxins.

Therapy for botulism intoxication primarily consists of supportive care, including mechanical ventilation, and passive immunization with an antitoxin, if available. Historically, human botulism mortality rates have been reported as high as 60%^{7,8}; however, with improved standards of care, such as respiratory support mechanisms and intensive care that includes antibiotics and antitoxins, mortality rates have decreased to < 7%.^{1,9} Although mortality rates have improved, duration of hospitalization and length of stay in intensive care units (ICUs) continue to present a major burden to the healthcare system, with hospital stays up to 6 months to recover from botulism.

Botulism antitoxin heptavalent (A, B, C, D, E, F, and G – Equine; BAT; Emergent BioSolutions Inc., Canada) is a sterile solution of F(ab')₂ and F(ab')₂-related antibody fragments prepared from plasma obtained from horses that have been immunized with a specific serotype of botulinum toxoid and neurotoxin. To obtain the final heptavalent product, the seven antitoxin serotypes are blended. In the United States, BAT product is indicated for the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin serotypes A through G in adults and pediatric patients (including infants). The mechanism of action of BAT product is through passive immunization with equine-derived polyclonal antibody fragments. In the United States, BAT product was granted licensure in 2013 under the “Animal Rule” (Title 21 Code of Federal Regulation (CFR) 601 Subpart H). The “Animal Rule” provides the US Food and Drug Administration (FDA) with a mechanism to grant marketing approval based upon adequate and well-controlled animal studies when the results of those studies establish that the biological product is reasonably likely to produce clinical benefits in humans. A single vial of BAT product is the adult human dose. Each single-use vial contains a minimum potency of 4,500 units (U) for serotype A antitoxin, 3,300 U for serotype B antitoxin, 3,000 U for serotype C antitoxin, 600 U for serotype D antitoxin, 5,100 U for serotype E antitoxin, 3,000 U for serotype F antitoxin, and 600 U for serotype G antitoxin. This is based on previously marketed and unlicensed equine-derived botulinum antitoxin products, which had comparable levels of antitoxin as well as the theoretical neutralizing capacity of historical exposure level in humans where BAT product is 2 to 19 times in excess of the estimated maximum.^{10–15} The effectiveness of the FDA-approved dose was confirmed through multiple animal efficacy trials (i.e., guinea pig (GP) and nonhuman primate (NHP) animal models) and clinical experience.^{16,17}

The data collected in the BAT product developmental stage was used to support the one-vial BAT product dose. The traditional drug development paradigm for efficacy testing of drugs such as BAT product against botulinum neurotoxin exposure in humans is unethical. We hypothesized that a translational dose scaling model-based approach can help justify dose labeling decisions for approval under the “Animal Rule.” Using the available data, to date, and such a strategy, a BAT product vial with the current specification for neurotoxin neutralization is expected to result in significant protection against all clinical signs in humans for seven neurotoxin serotypes A to G. This provides an ideal framework for demonstrating that translational modeling can be a valuable tool. The approach to identify the efficacious dose in humans was two-fold. First, population pharmacokinetic (PopPK) models of BAT product serotype A antitoxin were developed in GPs, NHPs, and healthy humans. Cross-species BAT pharmacokinetic (PK) parameters were then determined and applied to support selection of the human dose. Second, the translation of BAT product efficacy to humans needs to be understood. To accomplish this, BAT product exposure-response in animal models of botulinum intoxication relating BAT concentration and mortality is developed^{18,19} and translated to humans. The objective of this analysis was to develop these PK models and exposure-response models for BAT product to justify the dose selection.

METHODS

Pharmacokinetic software

PopPK modeling of BAT product in serum was performed with Phoenix (version 1.1; Certara, Princeton, NJ). Summary tables and figures and inferential statistics was performed with Phoenix (version 1.1) or similar software. Exposure-response modeling was performed with R (version 2.11.0; Open Source Software) and S Plus (version 8.1; Insightful Corporation, Seattle, WA).

Data set construction

PK and pharmacodynamic (PD) information for BAT product was collected in a total of five studies. The BAT product PK models were based on a total of 264 GPs, 42 NHPs, and 39 healthy adult human subjects. GPs were sampled at 10 minutes, 4, 8, 12, 24, and 48 hours, and 3, 5, and 8 days postdose following an i.v. injection of either 0.0320 mL/kg or 0.160 mL/kg of BAT product. GP samples were pooled at each timepoint, thus only one pooled sample per timepoint was available for the analysis. Rhesus macaque NHP blood samples were collected at 4, 8, 12, and 24 hours postdose (intoxicated animals) or at 0.5, 2, 4, 6, 8, 12, 18, and 24 hours, and 3, 6, 9, 12, 14, and 20 days (unintoxicated animals) following i.v. injection of 0.0160 mL/kg or 0.160 mL/kg of BAT product. Blood samples from human subjects were collected at 0.5, 4, and 8 hours and at 1, 3, 7, 14, 21, and 28 days postdose following i.v. infusion of 0.160 mL/kg or 0.319 mL/kg of BAT product.

The exposure-response models were constructed using clinical signs data from an NHP postexposure prophylaxis study¹⁹ and with data from a postexposure prophylactic efficacy study of BAT product¹⁸ when administered to GPs following an i.m. intoxication equivalent to four times the GP i.m. lethal dose (GPIMLD₅₀) of neurotoxin serotypes A to G (45, 85, 20, 57, 732, 250, and 532 mouse intraperitoneal lethal dose 50% (MIPLD₅₀)/kg, respectively).¹⁸ The NHP serotype A neurotoxin challenge dose was four times the NHP i.v. LD₅₀ (10⁴ MIPLD₅₀/kg). All intoxicated BAT product-treated NHPs and, depending on the neurotoxin serotype and BAT product dose, 90% to 100% of intoxicated BAT product-treated GPs survived, whereas all intoxicated

placebo-treated GPs and NHPs died. Although data against neurotoxin serotypes A, B, C, D, E, F, and G was available in GPs, and PK data for serotypes A, B, C, D, E, F, and G antitoxins was available in humans, only serotype A neurotoxin data was available in NHPs. In addition, note that the interaction (PK/PD) between BAT product and neurotoxin were only available in NHPs for serotype A.

All serum concentrations of BAT product below the lower limit of quantification (<LLOQ; 1,290 out of a total of 2,894) of the Mouse Neutralizing Assay (MNA)²⁰ were flagged and set to missing for the PopPK analysis. Missing values for the covariates were replaced by the median value calculated for the appropriate animal subset. For GP data, as serum samples were pooled at each timepoint prior to analysis and only one pooled concentration per timepoint was available for analysis, continuous covariates, such as mean body weight, replaced the value for that animal subset. There were no other sample exclusions or data extrapolations. Actual sampling times were used when available. Further methodological details may be found on the BAT product page on the FDA website.²¹

Population PK model development

PK models were developed using plasma concentration-vs.-time data for BAT product obtained from GPs, NHPs, and humans after i.v. administration. These determined the systemic parameters such as clearance and volume. The PK parameters allowing prediction of disposition of BAT product in GPs, NHPs, and humans, were assumed to be species-independent and were scaled according to a generalized Dedrick approach where disposition is allometrically related to the power of an animal's body weight, as follows²²:

$$CL_i = aBW_i^b$$

$$V_i = cBW_i^d$$

$$CLd_i = gBW_i^b$$

$$Vt_i = eBW_i^d$$

$$CLdt_i = jBW_i^b$$

$$Vdt_i = kBW_i^d$$

where CL = clearance, V = volume of distribution, BW = body weight, CLd = intercompartmental distribution, Vt = extravascular volume, CLdt = deep tissue intercompartmental distribution, Vdt = deep tissue extravascular volume and d = allometric exponents, and a, g, e, j, k, and c = typical values for a BW = 1 kg. Extravascular volume and deep tissue extravascular volume was required in order to describe the multiexponential decline in concentration following i.v. data. Intercompartmental and deep tissue intercompartmental clearance are similar to central clearance and blood flows with respect to the exponent b, whereas extravascular and deep tissue extravascular volumes are expected to scale similar to central volume. In this scenario, the exponents b and d can be compared to more generalized values accepted in the literature ($b = 0.75$ and $d = 1.0$).²² The model developed for serotype A antitoxin component of BAT product was used only as a benchmark for comparison with serotypes B through G antitoxin components of BAT product, as the number of compartments was not assumed constant across antitoxin serotypes. Models were evaluated using diagnostic plots of goodness of fit and the related statistical estimators: (i) minimum value of objective function (MOF) and (ii) the difference between MOF values of a reference PK model and a tested PK model (Δ MOF). Individual Bayesian PK parameters of BAT product (i.e., CL,

V_c , and V_p) were derived from the population analysis, and any secondary parameters (terminal half-life ($t_{1/2}$), area under the curve (AUC), and maximum plasma concentration (C_{max})) were derived from the primary parameters, either directly (e.g., $AUC = \text{dose}/CL$) or based on simulated profiles, when appropriate.

The determination of differences in PK between different subgroups of the population is critical to determining possible dose adjustments in those subgroups in a clinical setting. The various subpopulations were, therefore, characterized in terms of their defining covariates, and an evaluation of possible differences in PKs as a function of these covariates was undertaken. In a first step, the relationships between covariates and PK parameters of serotype A antitoxin component of BAT product were explored graphically to obtain preliminary information of covariates likely to affect the PKs of BAT product. Examples of the factors that were investigated included intrinsic factors, such as sex and species, and extrinsic factors, such as dose level and neurotoxin presence.

Exposure-response model development

Because typical assessment of BAT product efficacy in humans cannot be performed using traditional clinical trial methods, the efficacy of BAT product in humans at the proposed clinical dose needs to be determined in animals and then extrapolated to humans. It is expected that the interaction between BAT product and neurotoxin in the systemic circulation (i.e., the PDs) remains the same across species and, thus, combining the relationship between antitoxin concentration and effect in animals and estimated antitoxin concentration of BAT product in humans is sufficient to estimate efficacy for a specific antitoxin concentration in humans.

A key component of this assessment is the relationship between BAT product exposure and effect, which can be constructed using available animal data. Concentration levels were not estimated experimentally in animals in these efficacy studies; they were simulated using the PK model. Following simulation of the BAT product exposure (AUC) in animals that showed a range of clinical signs (from moderate to death) using the PK model developed above, logistic regression was used to explore the relationship between these BAT product exposure measures predicted by the PopPK model (AUC) and the probability of survival, as well as AUC from the PK model vs. relevant moderate clinical signs.^{23,24} First, the frequency of occurrence of each clinical sign was compiled by neurotoxin serotype and sign from postexposure prophylaxis studies. Second, the regression was performed and parameter estimates (logit scale) for the regression model (slope and intercept) were obtained for each exposure-response relationship. The regression line also included a measure of its precision (confidence intervals (CIs)) and a *P* value. Figures of observed and fitted probabilities of response as a function of BAT product exposure were derived. Logistic regressions of response were analyzed in all populations. The PK/PD correlation analysis was performed during the treatment phase of the study (day 1). Response with a covariate designating time of onset was not evaluated.

All animals that showed moderate clinical signs were included in the exposure-response analysis. Severe clinical signs were not included as they were considered bracketed between the moderate signs above and survival rates. A correspondence was made between GP and NHP clinical signs. GP salivation, lacrimation, noticeable change in breathing pattern or rate, and hind limb local paralysis/weak limbs corresponded to the NHP oral discharge, respiratory distress, and muscular weakness, respectively. There was no direct correlation in NHPs to "lacrimation," therefore this category included only GPs. This was consistent with NHPs not displaying any other moderate clinical signs.

BAT unit dose risk assessment

Ultimately, the question being asked is the following: Given an exposure to neurotoxin, how much risk (of death, weak limbs, etc.) am I exposed to if I am given $1 \times$ dose of BAT product?

The actual potency of BAT product will vary from lot-to-lot, although all studies contained in the analysis were based on the same certificate of

analysis and used the actual potency in development. One unit (U) of BAT product neutralizes 10,000 MIPLD₅₀ units of botulinum neurotoxin (all neurotoxin serotypes except neurotoxin serotype E, where specificity is 1 U = 1,000 MIPLD₅₀); therefore, a typical (1× vial) administration would result in 104 million MIPLD₅₀ neurotoxin serotype A units neutralizing capacity and 258.5 million MIPLD₅₀ units neutralizing capacity across neurotoxin serotypes.

Based on preliminary information, a human clinical dose of 11.17 mL (1 vial) was proposed and needs to counter serum neurotoxin levels of at least 32–160 MIPLD₅₀/mL for serotypes A, B, C, D, E, F, and G, based on historic intoxication instances. The highest levels of neurotoxin ever reported in a US patient with foodborne botulism was 32 MIPLD₅₀/mL (neurotoxin serotype unspecified),²⁵ whereas 160 MIPLD₅₀/mL was the highest level of neurotoxin ever reported for a patient diagnosed with foodborne botulism due to serotype E in the United Kingdom.²⁶ In a case of cosmetic injection of an unlabeled highly concentrated botulinum neurotoxin preparation (serotype A), a concentration of 12 to 24 MIPLD₅₀/mL of neurotoxin was reported.²⁷ The $t_{1/2}$ of neurotoxin is difficult to determine due to its intrinsic toxicity, which kills the animals quickly. In rodents, concentrations of neurotoxin have been reported to decline in a mono-exponential manner following an i.v. injection, with a $t_{1/2}$ of ~4 hours.²⁸

We, therefore, leveraged modeling and simulation to determine whether a BAT product dose of 11.17 mL would result in high enough systemic exposure to counter systemic effects of neurotoxin serotypes, based on the protection that BAT product bestows on the animals exposed to lethal levels of neurotoxin.

If we define a given BAT product concentration as a benchmark for what we consider to be “negligible” risk to neurotoxin exposure, then it stands to reason that any exposure to BAT product greater than this benchmark would be an “acceptable” dose of BAT product, because the exposure was greater than this benchmark, which was already associated with “no-risk.” We can refer to this ratio between BAT product exposure and a defined efficacy benchmark as the efficacy margin (EM = $AUC_{\text{human dose}}/AUC_{\text{MEE}}$, where MEE is the minimum effective exposure). The magnitude of this BAT product antitoxin concentration to benchmark ratio would provide an assessment of the “safety” margin. A ratio of 1,000 indicates we are above the negligible risk benchmark and our risk is essentially 0. A ratio of 1 indicates we are very close to the benchmark and essentially have no exposure “buffer.” A ratio < 1 indicates that we are no longer in a zone of negligible risk. This ratio, therefore, provides a rapid assessment of the risk related to a 1× dose of BAT product. To derive this ratio, the following must be known: (i) the benchmark value of antitoxin concentration of BAT product that will constitute minimal risk of an adverse event following neurotoxin exposure, and (ii) the actual antitoxin concentration expected in humans following a 1× dose of BAT product.

In this study, the antitoxin concentration of BAT product that would result in 80% survival was arbitrarily chosen and identified as the benchmark MEE that is used in the ratio for assessing risk. Similar comparisons to higher benchmarks (e.g., 90%) were also made (data not shown). The human antitoxin concentration corresponding to administration of 11.17 mL (1 vial) or 22.34 mL (2 vials) of BAT product can, therefore, be compared to the MEE and an EM can be estimated.

RESULTS

Population pharmacokinetic analysis of BAT product following dosing in guinea pigs, nonhuman primates, and human subjects

PopPK analysis of BAT product for all antitoxin serotypes were best fitted using a three-compartment model, with the exception of serotype E antitoxin component of BAT product, which was fitted using a two-compartment model. This different behavior can be explained by the low peak concentrations of the serotype E antitoxin profiles relative to the LLOQ (Figure 1). The apparent two-compartment model behavior was due to a lack of information (i.e., drop to <LLOQ at the terminal phase). It is speculated that a higher dose of BAT product antitoxin serotype E could have resulted in higher systemic exposure and, consequently, a better assessment of the terminal elimination phase of the product.

The PopPK parameters of BAT product derived for all BAT product antitoxin serotypes are summarized in Table S1. Mean systemic clearance ranged from 4.44 mL/h/kg (serotype D antitoxin) to 14.75 mL/h/kg (serotype A antitoxin) and the volume of distribution at steady-state (V_{ss} , the sum of V_c , V_p , and V_{dt}) of BAT product ranged from 4.44 mL/kg (serotype E antitoxin) to 1,309 mL/kg (serotype C antitoxin). The total V_{ss} (the sum of V_c , V_p , and V_{dt}) was comparable to known volumes of serum (42.9 mL/kg for serotype D antitoxin) and water (600 mL/kg for serotype B antitoxin) in humans.²⁹ The different PK parameter values across antitoxin serotypes are not unexpected given the nature of the BAT product F(ab')₂ and F(ab')₂-related antibody fragments. Renal and nonrenal pathways both account for the elimination of protein therapeutics,³⁰ with overall clearance and distribution being related to molecular size and charge.^{30,31} Because BAT product is composed of F(ab')₂ fragments of ~100 kDa and Fab fragments of ~50 kDa, however, the contribution of renal clearance is expected

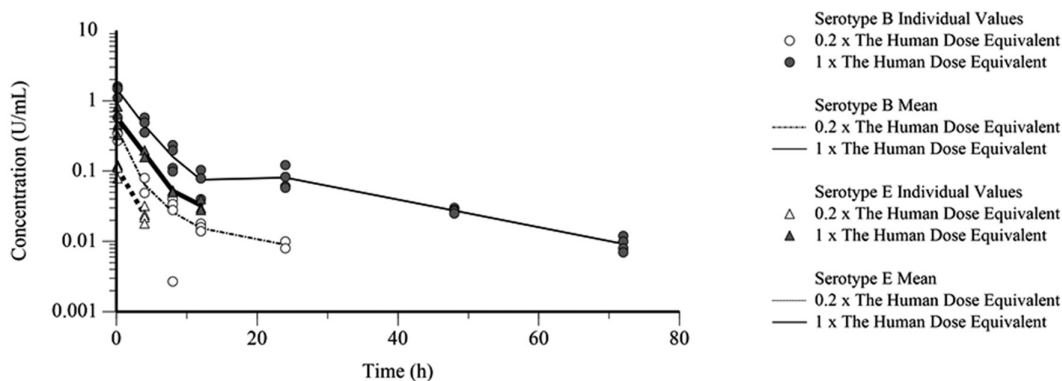


Figure 1 Comparison of profiles of BAT product antitoxin serotypes B and E in guinea pigs. Filled symbols represent observed pharmacokinetic (PK) values following a high dose of BAT product, whereas empty symbols represent observed PK values following a low dose of BAT product. Lines represent mean data. BAT, botulism antitoxin heptavalent.

to be minimal.³⁰ Age-related effects on the PKs of BAT product are being investigated as part of clinical trial (ClinicalTrials.gov, NCT02051062) with corresponding recommendation for dosing. Due to the data currently available, these effects could not be investigated as part of the current framework. Corresponding allometric exponent values were close to 0.75 or 0.66 in all cases except for serotype D antitoxin clearance, which was significantly lower (0.376). This indicated that changes in exposure from animals to humans (i.e., human to animal ratios) were different with each BAT product antitoxin serotype and that antitoxin serotype ratios of exposure (serotypes A to G antitoxin clearances) within the GPs were not representative of ratios of exposure for the same antitoxin serotypes within humans. Volume slope estimates were close to 1 for all serotypes except for serotypes A and D antitoxins, where slopes were significantly different than 1 (0.460 for serotype A and 0.193 for serotype D antitoxins). BAT product antitoxin serotypes B through G allometry was limited to two species; however, results indicated that using a slope of 0.75 for CL and 1.00 for volumes,²² in the absence of other data, would be a reasonable alternative to empirical scaling given that the 95% CI on the above slopes often include the value of 0.75 for CL and 1 for volumes. For these types of protein fragments, as well as large molecules, use of scaling factors of 0.75–0.9 have often been reported.^{30,32–35}

PK interaction of BAT product and neurotoxin serotype A

The determination of differences in PK between neurotoxin-naïve (prophylactic) and non-naïve (therapeutic) subjects is critical to determining possible dose adjustments in those subgroups in a clinical setting. In the case of non-naïve subjects, differences in the PKs of BAT product would result from the interaction between BAT product and its ligand neurotoxin.

Potential PK interactions between the neurotoxin and the antitoxin were, therefore, evaluated. Relationships between BAT product and neurotoxin were explored graphically to obtain preliminary information on the effect of neurotoxin on the PK of BAT product. This analysis was performed considering that clinical trials can only assess the PK of BAT product in neurotoxin-free subjects; whereas, BAT product treatments would be

expected to be administered following possible systemic exposure to neurotoxin. Preliminary analysis of the potential PKs of BAT product in presence of neurotoxin in NHPs is presented in **Figure 2**.

Note that the neutralizing assay that quantifies the level of BAT product in plasma measures the “free” form of BAT product, and not total (“free + bound to neurotoxin”) BAT product. Based on the above concentration-time profiles, the presence of neurotoxin appeared to result in a slower terminal elimination of unbound BAT product. The potential impact of neurotoxin on BAT product PKs was formally tested for serotype A neurotoxin in NHP data. The interaction was formally evaluated using nonlinear mixed effects by a stepwise forward additive approach using a *P* value of 0.05 (Δ -2LL of 3.84) and a backward elimination using a *P* value of 0.01 (Δ -2LL of 6.635; Δ -2LL values corresponding to a chi-square distribution with one degree of freedom at the appropriate power level), when administration of BAT product followed neurotoxin challenge. The extent of PK changes of BAT product when in the presence of neurotoxin were quantified using the following equation, where CF is the correction factor that will alter the BAT product PK when neurotoxin is present:

$$PK = tvPK + CF_{PK(\text{Neurotoxin} = \text{True})}$$

There was a significant effect only on intercompartmental distribution clearance. The resulting effect of neurotoxin on antitoxin half-life is presented in **Table 1**.

Exposure-response models of survival and other clinical signs

Logistic regression was used to explore the relationship between BAT product exposure measures predicted by the population PK model (AUC) and the probability of survival. Parameter estimates (logit scale) for the regression model (slope and intercept) were estimated for each exposure-survival relationship.

A summary of logistic regression parameters for survival is presented in **Table S2**. The statistical significance of the exposure-survival relationship for the predicted probability of survival was tested at an alpha level of 0.05. For serotype A neurotoxin, where there were the most data available, the AUC effect on survival was

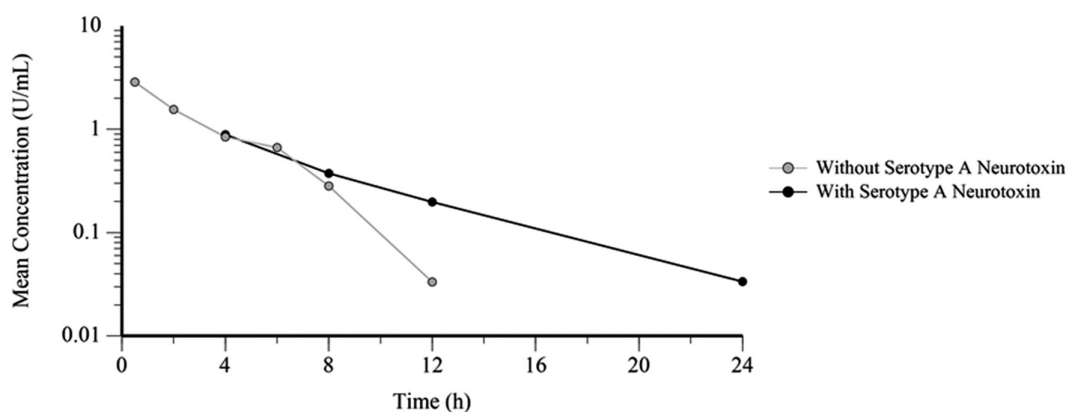


Figure 2 Mean concentration of BAT product antitoxin serotype A in nonhuman primates (NHP) with and without neurotoxin. Filled symbols represents the prolongation of half-life observed when BAT product is administered in presence of neurotoxin serotype A. BAT, botulism antitoxin heptavalent.

Table 1 Effect of botulinum neurotoxin on NHP PK parameters of antitoxin (BAT) product

| Treatment | $t_{1/2\alpha}$ (h) | $t_{1/2\beta}$ (h) | $t_{1/2\gamma}$ (h) | %AUC $_{\gamma}$ |
|--------------------------|---------------------|--------------------|---------------------|------------------|
| BAT product | 0.227 | 1.6 | 6.00 | 67.7 |
| BAT product + neurotoxin | 0.907 | 4.5 | 20.0 | 8.73 |

%AUC $_{\gamma}$, proportion of the area under the curve that is represented by the terminal phase; BAT, botulinum antitoxin heptavalent; NHP, nonhuman primate; PK, pharmacokinetic; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, intermediate half-life; $t_{1/2\gamma}$, terminal half-life.

highly significant, suggesting a direct relationship between BAT product levels and the probability of survival in GPs and NHPs.

The neurotoxin level was linked to clinical severity, with the highest BAT product level required to prevent the most severe clinical outcome of lethality. Given typical disease progression, lower levels of neurotoxin were expected to result in more moderate, nonlethal, clinical signs, such as muscle weakness and signs of respiratory distress. These would be expected to correspond to typical early clinical signs of intoxication in humans, which can include drooping eyelids, difficulty swallowing, slurred speech, and shortness of breath. To investigate the risk of occurrence of these potential outcomes in humans, similar analyses as presented for survival were performed. As such, the relationship between the antitoxin concentration of BAT product and a total of four moderate clinical signs considered related to neurotoxin administration from GP efficacy studies (i.e., salivation, lacrimation, weak

limbs, and noticeable change in breathing sounds, rate, or pattern) were evaluated using logistic regression models. These were based on frequency, as they occurred during the pivotal study. Insufficient data were available to construct the logistic regression model for salivation/lacrimation, as such the exposure-response model did not minimize successfully.¹⁸ Sufficient data and an appropriate model were available to construct logistic regressions for other neurotoxin serotypes (D and E). Results are presented in **Table S3**.

Risk analysis and calculation of the EM: Survival to neurotoxin

The relationship between the antitoxin concentration of BAT product serotype A and survival derived with the logistic regression model is presented in **Figure 3**. In this figure, individual animals (empty symbols) are either assigned the value of 1 or 0 depending on their survival at the end of the studies. These are then plotted against the AUCs for these animals (obtained from the PK model). The ratios of surviving animals over the total number of animals result in the observed probability of survival for particular ranges of observed AUCs (filled circles). The logistic regression models the probability of survival vs. the simulated AUC of BAT product (filled triangle). Based on these regression results, a very steep relationship was observed between the probability of survival and the antitoxin concentration of BAT product for serotype A as 80% survival was predicted to occur at a relatively low AUC of BAT product.

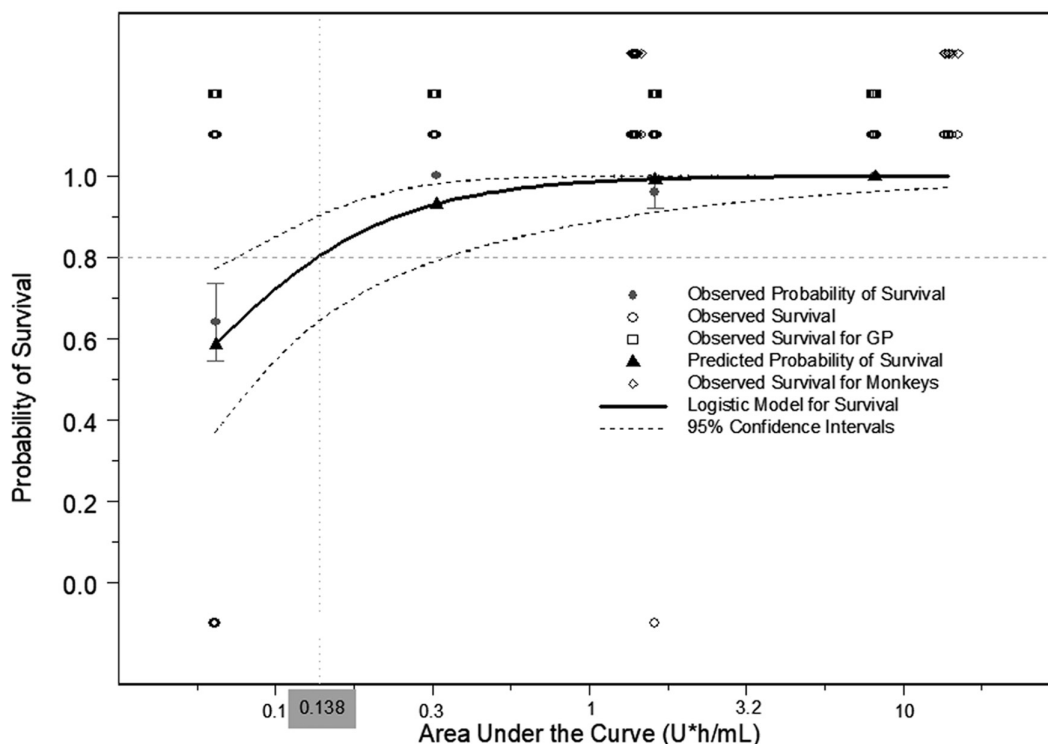


Figure 3 Logistic regression of antitoxin concentration of BAT product serotype A vs. survival. Black symbols represent individual observed data (0 for not survived; 1 for survived). Grey symbols represent observed probability composite from the individual data. Black line represents model fit of the probability data, whereas black dotted line represents confidence interval. Grey dotted lines represents the 80% survival reference (target survival rate) leading to the area under the curve that is defined as the minimum efficacious exposure (MEE). See Emanuel *et al.*¹⁸ and Kodihalli *et al.*¹⁹ for study details and a description of the experimental data used in the regression analysis. Probabilities of 0 and 1 were offset to improve data visualization. GP, guinea pig.

Table 2 The lower and upper 95% CIs of these predicted AUCs in GPs and NPs, as well as their corresponding predicted probabilities as a function of antitoxin (BAT) product serotype A exposure

| Survival probability | | BAT product AUC (U*hour/mL) | |
|----------------------|--------------------|-----------------------------|--------------------|
| Predicted | 95% CI lower-upper | Predicted | 95% CI lower-upper |
| 58.3% | 36.8–77.0% | 0.064 | 0.034–0.12 |
| 70.3% | 52.1–83.7% | 0.094 | 0.054–0.164 |
| 80.1% | 64.1–90.0% | 0.137 | 0.076–0.246 |
| 90.2% | 75.6–96.5% | 0.249 | 0.114–0.545 |
| 95.1% | 81.9–98.8% | 0.429 | 0.15–1.226 |
| 99.0% | 90.3–99.9% | 1.415 | 0.25–7.996 |

AUC, area under the curve from 0 to infinity as derived from the logistic regression models; BAT, botulism antitoxin heptavalent; CI, confidence interval; GP, guinea pig; NHP, nonhuman primate. Corresponds to the minimum efficacious exposure (MEE) when survival = 80%.

The developed logistic regression model (Table S2) can then be used to predict antitoxin concentrations at various probabilities of survival. The lower and upper 95% CIs of these predicted AUCs in GPs and NHPs, as well as their corresponding predicted probabilities, are presented in Table 2. The point estimate AUC associated to an 80% survival rate was therefore 0.137 U*hour/mL, which is our definition of the MEE. The lower 95% CI of the observed AUC (conservative estimate) in the human PK study (1× vial, with the current vial specifications), as estimated by noncompartmental analysis, was ~ 21.0 U*hour/mL. Overall, the resulting EM (based on antitoxin concentration across 2 species) for neurotoxin serotype A is 153 ($AUC_{human}(1x\ dose)/AUC_{MEE} = 21.0/0.137$) for the proposed clinical dose of 1 vial of BAT product derived at 104 (NHP)/kg and 18 MIPLD₅₀/guinea pig or 40 MIPLD₅₀/kg neurotoxin exposure (Table 3).

Table 3 EMs for 80% survival of neurotoxin serotypes A to G in humans

| Neurotoxin serotype | BAT product | | EM |
|---------------------|-----------------|---|-------|
| | MEE (U*hour/mL) | AUC _{human} (lower 95% CI) (U*hour/mL) | |
| A | 0.137 | 20.96 | 153.0 |
| B | 0.085 | 17.44 | 205.2 |
| C | 0.249 | 23.88 | 95.9 |
| D | 1.413 | 4.15 | 2.9 |
| E | 0.794 | 5.25 | 6.6 |
| F | 0.072 | 18.54 | 257.5 |
| G | 0.088 | 4.86 | 55.2 |

MEE (area under the curve; AUC) in animal species as derived from the logistic regression models. Corresponds to 80% survival except for neurotoxin serotypes B, F, and G, which correspond to more conservative 85%, 82%, and 89% survival probabilities due to lack of quantitative data at lower probabilities. See Table 2 for how the MEE was derived for toxin serotype A. AUC_{human}, area under the curve observed in healthy volunteers; BAT, botulism antitoxin heptavalent; CI, confidence interval; EM, efficacy margin; MEE, minimum efficacious exposure.

Risk analysis: protection of BAT product from moderate clinical signs

Based on results presented in Table S3 in GPs and NHPs, a relatively shallow relationship was observed between the probability of seeing a change in the appearance of weak limbs and the antitoxin concentration of BAT product for serotype A neurotoxin. Thus, protection from BAT product appeared to occur at a relatively high AUC, although higher antitoxin concentration of BAT product did result in lower probability of weak limbs for serotype A neurotoxin. On the other hand, a very steep relationship was observed between the probability of seeing a change in breathing sounds, rate, or pattern and the antitoxin concentration of BAT product for serotype A neurotoxin. Thus, lower doses of BAT product were required for protection. Logistic regression models of moderate clinical signs were driven with the human-specific antitoxin concentration data following administration of 1× BAT product and EM values were estimated. Efficacy margin in humans for all serotypes are summarized in Table 4. As with lethality, where the objective was 80% survival, efficacy was evaluated when there was 80% protection from occurrence of moderate clinical signs (thus 20% occurrence).

Efficacy margin for salivation signs ranged from 1.71 to > 238 across all neurotoxin serotypes following administration of 1× dose of BAT product. Similarly, EM for lacrimation signs ranged from 1.89 to > 238 across all neurotoxin serotypes. Overall, the administration of 1× dose of BAT product is expected to result in significant protection against salivation and lacrimation signs for all neurotoxin serotypes. It should be noted that for cases where logistic regression was not useful (due to lack of clinical signs), an arbitrary MEE value (AUC < 0.1 U*hour/mL) was used to estimate a lower bound of antitoxin concentration of BAT product. Efficacy margin values for “weak limb” clinical signs ranged from 0.26 to 51.22 across all neurotoxin serotypes following administration of 1× dose of BAT product. The EM of BAT product for serotype E neurotoxin (0.26) should be interpreted with caution because the 20% probability of weak limbs was not included within the range of probabilities that was used to construct the exposure-response model, because the highest dose of BAT product resulted in only 23.6% of probability of weak limbs. Nevertheless, it should be noted that survival based on this antitoxin concentration of BAT product and serotype E neurotoxin would be close to 99.9%. The logistic regression model may be used to extrapolate the antitoxin concentration associated to the 20% probability benchmark for “weak limb.” This exploratory analysis suggests that 4.5× BAT product would be associated with 20% occurrence of weak limbs for serotype E neurotoxin. Efficacy margin values for “Noticeable Change in Breathing Sounds, Rate, or Pattern” signs ranged from 0.26 to 88.81 across all neurotoxin serotypes following administration of 1× dose of BAT product.

DISCUSSION

Overall, based on the current data available on BAT product, the probability of survival for serotypes A, B, C, D, E, F, and G neurotoxin following a dosing of 1× BAT product were at least 99.9%, 95.9%, 98.0%, 99.0%, 99.9%, 99.6%, and 98.7%, respectively.

Because the antitoxin concentration of BAT product following administration of 1× and 2× vials in humans was 21.0 and

Table 4 EM for 20% probability of moderate clinical signs following neurotoxin serotypes A to G administrations in humans

| Clinical signs | Neurotoxin serotype | BAT product | | |
|---|---------------------|--|-----------------------------------|--------|
| | | MEE (U*hour/mL) for 20% probability | AUC (lower 95% CI) (U*hour/mL) | EM |
| Salivation | A | < 0.1 ^a | 20.96 | > 210 |
| | B | < 0.1 ^a | 17.44 | > 174 |
| | C | < 0.1 ^a | 23.88 | > 238 |
| | D | 2.42 | 4.15 | 1.71 |
| | E | 0.42 | 5.25 | 12.47 |
| | F | < 0.1 ^a | 18.54 | > 185 |
| | G | < 0.1 ^a | 4.86 | > 48.6 |
| Lacrimation | A | < 0.1 ^a | 20.96 | > 210 |
| | B | < 0.1 ^a | 17.44 | > 174 |
| | C | < 0.1 ^a | 23.88 | > 238 |
| | D | 2.20 | 4.15 | 1.89 |
| | E | 0.76 | 5.25 | 6.94 |
| | F | < 0.1 ^a | 18.54 | > 185 |
| | G | < 0.1 ^a | 4.86 | > 48.6 |
| Weak Limbs | A | 0.97 | 20.96 | 21.65 |
| | B | 4.11 | 17.44 | 4.25 |
| | C | 0.98 | 23.88 | 24.29 |
| | D | 3.77 | 4.15 | 1.10 |
| | E | 19.87 ^b | 5.25 | 0.26 |
| | F | 0.36 | 18.54 | 51.22 |
| | G | 0.35 | 4.86 | 14.01 |
| Noticeable Change in Breathing Sounds, Rate, or Pattern | A | 0.24 | 20.96 | 88.81 |
| | B | 0.34 | 17.44 | 51.90 |
| | C | 0.89 | 23.88 | 26.80 |
| | D | 4.16 | 4.15 | 1.00 |
| | E | 19.87 ^b | 5.25 | 0.26 |
| | F | 0.49 | 18.54 | 38.15 |
| | G | 0.57 | 4.86 | 8.57 |

MEE, (AUC) in animal species as derived from the logistic regression models.

AUC_{human} = area under the curve observed in healthy volunteers; BAT, botulism antitoxin heptavalent; CI, confidence interval; EM, efficacy margin; MEE, minimum efficacious exposure.

^aMEE value fixed to < 0.1 U*hour/mL due to lack of clinical signs. ^bMEE value fixed to 19.87 U*hour/mL (higher dose level of BAT product) due to lack of quantitative data to interpolate a 20% probability.

87.7 U*h/mL (EM of at least 153 to 640), respectively, more than 99.9% survival against serotype A neurotoxin would be predicted for the 1× vial or the 2× vial dose. Information on the effects of serotype A neurotoxin is available in both GP and NHP animal models and learnings from this serotype neurotoxin can be applied to other serotype neurotoxins assuming similar translatability. As such, the EM from this serotype neurotoxin can be interpreted in a number of different ways. For example, an EM of 153 gives the flexibility to halve the administered dose (0.5×), in times of product shortages, and still be ~ 75× greater than the 80% efficacy benchmark. Alternatively, a subcutaneous (s.c.) administration of BAT product with a hypothetical low bioavailability down to 1% (from the 100% current i.v. administration) would still be 1.5× greater than the 80% survival efficacy benchmark for serotype A neurotoxin. Similarly, this margin can

provide a safety margin for any potential decrease in BAT product antitoxin concentration due to increases in neurotoxin dose and any possible interaction between the two internal doses of BAT product and neurotoxin. Furthermore, by dividing the proposed human dose (1×) by the EM (1/153 = 0.0065x), we find the human dose that will protect 80% of the population. Because BAT product is expected to be in excess of neurotoxin, there is currently no evidence that increasing doses of neurotoxin would alter the EM at least until units of neurotoxin exceed the ~ 100 million MIPLD₅₀ neutralizing units of antitoxin serotype A available in the systemic circulation following the proposed clinical dose of BAT product. As the antitoxin/neurotoxin complex is inactive, additional clinical signs would not be expected to manifest until the free fraction of BAT product significantly decreases, and that would occur when concentrations of neurotoxin exceed

the ~ 100 million MIPLD₅₀ neutralizing units of serotype A antitoxin component of BAT product.

A similar analysis was undertaken with serotypes B to G neurotoxin. The EM was estimated in the same manner as for serotype A neurotoxin with the exception that levels of BAT product were only available in GPs. The EM for serotypes A to G neurotoxin are summarized in **Table 3**. BAT product offered the greatest protection for serotypes B and F neurotoxin following 1× dose of BAT product, with EM values of 205.2 and 257.5, respectively. On the other hand, greater lethality was observed for serotypes D and E neurotoxin, which resulted in a decreased protection for a 1× dose of BAT product. The greater toxicity of these neurotoxin serotype neurotoxin was reflected in the observed data,^{18,19} where serotype D and E neurotoxin displayed the lowest survival rates of the seven serotypes (53% and 72%, respectively). As such, the EM for serotype D and E neurotoxin (2.9 and 6.6, respectively) were at least eight times lower than the next lowest EM (55.2 from serotype G neurotoxin). For example, a 33% decrease in BAT product dose or exposure (due to variability) would possibly result in an unacceptable loss of efficacy for serotype D and E neurotoxin as protection of BAT product from these neurotoxins would possibly fall below 80%.

For moderate clinical signs, the EM of BAT product for serotype E neurotoxin (0.26) should be interpreted with caution because the 20% probability was not included within the range of probabilities available to construct the exposure-response model, as only 71.4% of animals displayed noticeable change in breathing sounds, rate, or pattern at the highest dose. Because the exposure-response relationship for “noticeable change in breathing sounds, rate, or pattern” was not well-characterized based on data currently available, an extrapolation to a 20% probability of event was not performed. Additional data are needed to further characterize the shape of this exposure-response relationship at higher dose levels of BAT product for serotype E neurotoxin.

Of course, the presence of neurotoxin itself results in a marked effect on the $t_{1/2\gamma}$ of BAT product, something that the source of our reference AUC, the human PK studies does not and cannot consider. On the other hand, this effect is expected to result in a minor impact on total drug exposure because the terminal elimination phases of BAT product only accounted for 9% of the total AUC (**Table 2**) when neurotoxin is present. Furthermore, systemic CL and volumes are not significantly impacted by the presence of neurotoxin as demonstrated by a systematic investigation of the effect of neurotoxin on the NHP PK parameters, supporting the statement that such a finding would be of minimal clinical relevance and would not have any significant impact on exposure metrics, such as AUC and C_{max} . At most, the $t_{1/2\gamma}$ of BAT product when in the presence of neurotoxin may result in a slightly higher minimum concentration (C_{min}), as compared with BAT product in neurotoxin-free humans, due to the increase in $t_{1/2}$. However, additional data are required to assess the effect of different dose levels of neurotoxin on PK parameters of BAT product in animals to further understand the dose-effect relationship on PK parameters of BAT product. Furthermore, it should be considered that the underlying exposure of neurotoxin is difficult to determine due to its intrinsic toxicity, which kills the animals quickly. However, as opposed to other bioterror threats, such

as anthrax, BAT product efficacy vis-à-vis neurotoxin can be verified in a clinical setting. Using the available data to date; however, a BAT product vial with the current specification for neurotoxin neutralization is expected to result in significant protection against all clinical signs in humans for seven neurotoxin serotypes A to G.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

D.A., D.B., S.K., C.H., and J.S.R. are currently employees and stockholders of Emergent BioSolutions. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

M.B. and J.R. wrote the manuscript. M.B., D.B., S.K., C.H., and J.R. designed the research. D.B., S.K., and C.H. performed the research. D.A., D.B., S.K., E.S., C.H., and J.R. analyzed the data.

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