Antitoxin therapy for botulinum intoxication
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Botulinum toxin is the most toxic substance known to mankind. It is involved in infant botulism, classical food-borne botulism, wound botulism, and is a threat in biological warfare or bioterrorism. The only successful drug currently available to treat botulinum intoxication is equine antitoxin, and there is potential for much improvement of this product. This review discusses the factors that could be changed to improve the safety and efficacy of botulinum antitoxins. The host species could be changed from horses to other animals such as sheep or goats, in order to lower the immunogenicity of the antiserum. The choice of immunogen is extremely important in improving the potency of the product. Fractionation of the antitoxin and affinity purification are two further ways in which the safety and potency of the product could be increased. The current botulinum antitoxin assay has a lethal endpoint, and several more humane assays for antitoxin are discussed. The dosing level for botulinum antitoxin is excessively high when the amount of toxin involved in intoxication is so small. Optimising the dosage of antitoxin would further improve the safety of the product. The therapeutic window of the current product is also discussed, along with potential mechanisms of increasing the therapeutic window.

INTRODUCTION
Botulinum toxin, produced by the Gram-positive bacteria Clostridium botulinum, C. argentinense, C. baratii and C. butyricum, is widely acknowledged to be the most toxic substance known to mankind. It can cause severe incapacitation and death in the smallest dose, with an estimated human lethal dose of less than 100 pg toxin/kg body weight [1]. Although botulinum toxin is usually only ingested when eating contaminated food, its extreme toxicity has made it a substance of choice for bioweapons, being cheap, easy to produce in large quantities, and relatively stable when stored in the correct form [2,3]. Botulinum toxin is the only non-replicating agent amongst the six biological agents considered to be of greatest threat to civilians [4]. The risk of botulinum toxin being used as a weapon is no longer theoretical; both the Aum Shinriko cult [5] and the Iraqi biological warfare programme [6] are known to have produced large quantities of botulinum toxin for offensive purposes.

Although a vaccine against botulinum toxin is available for laboratory workers, the only effective post-exposure therapy (and the only defence against bioterrorist attack) is botulinum antitoxin. Botulinum antitoxin consists of a purified fraction of immunoglobulins prepared...
from a hyperimmunised animal; unfortunately botulinum antitoxins often show undesirable side-effects. This review is intended to summarise the current methods of antitoxin production and therapy, as well as discussing some of the methods available for improving the efficacy and safety of botulinum antitoxins.

**TOXIN PRODUCTION**

The most common form of botulism is foodborne, occurring when *C. botulinum* grows in insufficiently preserved foodstuffs. The bacteria can also flourish in the gut of young infants due to a lack of competing flora, causing infant botulism, or very rarely in wounds (often caused by intravenous drug abuse), causing wound botulism [7]. In each case the results of intoxication are similar, although initial symptoms may vary due to the local effects of toxin production. The *Clostridium* spp. that produce botulinum toxin are spore-forming obligate anaerobes, and they release the toxin on bacterial lysis. Although the toxin is heat labile – it is destroyed by a few minutes treatment at temperatures above 80°C, spores are able to survive for longer than 1 h when treated at 120°C [8], leading to contamination of improperly prepared foods.

The toxin can be classified into seven serotypes, A–G, each of which can be neutralised only by its specific antiserum. Types A, B, E and F are common causes of human botulism, with types C and D being more common in botulism of animals [9]. No cases have been attributed to type G botulism [10]. The toxin is synthesised as a 150 kDa polypeptide chain, which is activated by protease cleavage to give a 100 kDa heavy chain (containing the domains involved in cell binding and endocytosis) joined by a disulphide bond to a 50 kDa light chain (containing the domains involved in proteolysis of the target proteins). The toxin can be released in either an active, cleaved form (type A, C, D and some types of B and F) or can be activated by cleavage by an exogenous protease (type E, and some type B and F).

**SYMPTOMS AND MECHANISM OF INTOXICATION**

Botulinum toxin acts as a neurotoxin, inactivating motor end plates and eventually producing a flaccid paralysis. Botulism is characterised by a symmetrical descending weakness; as the bulbar musculature is the most densely innervated and is well supplied with blood, symptoms appear here first [11]. The first symptoms are usually a flaccid paralysis of the muscles of the face, head, mouth and throat. This leads to a variety of bulbar symptoms including diplopia, mydriasis leading to blurred vision, photophobia, ptosis, dry mouth, dysphagia, dysarthria and dysphonia. Food-borne botulism may present additional symptoms due to the local effects of the toxin, such as nausea, vomiting, diarrhoea or constipation. If the toxin is present in a sufficiently high concentration a descending paralysis continues, with symmetrical skeletal muscle weakness and paralysis, eventually leading to death due to respiratory arrest. The onset of symptoms of human botulism usually occurs 18–36 h after exposure (range, 6 h to 8 days [12]), although it is difficult to detect early symptoms in laboratory animals. The speed of symptom development and severity of the symptoms are dependent on the dose received; a larger dose causes more severe symptoms to develop quickly in monkeys [13].

Botulism toxin is a relatively large protein, approximately the same size as an IgG molecule. Consequently it is unable to pass through the plasma membrane and gains access to the circulation by transcytosis [14]. In the case of food-borne botulism, lysis of bacteria releases active toxin, or progenitor toxin that is probably activated immediately by exogenous proteases. The active toxin is then transported across the epithelium of the stomach and small intestine [15] by transcytosis, releasing the toxin into the blood and lymph. Presumably a similar process occurs when the toxin is absorbed from the lungs after aerosol exposure. The toxin is finally distributed throughout the body by the blood to motor neurons where it has its lethal effect. Botulism neurotoxin binds to a variety of cell membranes, although at physiologically toxic concentrations it will bind only to the membrane of a motor neuron. It is likely that gangliosides are the target of the binding domain of the toxin [16]. Once bound the toxin is internalised by endocytosis [17], using a binding site on the heavy chain of the toxin, and the light chain (protease) is released into the cytoplasm.

The light chain of botulinum toxin is a zinc-dependent protease with an extremely high
specificity for its target. The targets vary between serotypes; but all are components of the pathway leading to synaptic vesicle release [18]. When these proteins are cleaved by botulinum toxin the synaptic vesicles cannot fuse with the plasma membrane, and accumulate at the presynaptic membrane. The blockage of acetylcholine release prevents activation of the motor end plate by the motor neuron, and causes the flaccid paralysis characteristic of botulism. Once the toxin has cleaved the synaptic vesicle docking proteins, recovery of the synapse is impossible, leading to degeneration of the neuron. In cases where recovery from botulinum intoxication occurs, new neurons are seen to sprout and re-innervate the muscle end plate [19]. This is an extremely slow process, after which a degree of residual weakness remains for a considerable length of time [20].

**THERAPIES FOR BOTULINUM INTOXICATION**

Despite a number of alternative therapies that have been tested in experimental and clinical models, the only effective therapy is the use of antitoxin. Various drug antagonists (including guanidine, aminopyridine and chloroquine) are able to delay the onset of paralysis by 1–2 h, but do not protect against the lethal effect of the toxin [7]. Studies on the efficacy of botulinum antitoxin have been carried out in only animals because of ethical considerations. The efficacy of antitoxin in humans is debatable, as no clinical study has been completed. In a retrospective study [21], patients who received antitoxin had a shorter illness and a lower fatality rate compared with those who did not receive antitoxin. These observations suggest that treatment with antitoxin reduces the period of hospitalization of a patient, although the therapeutic window is not known. Human botulinum immune globulin is currently under trial to treat infants with suspected infant botulism [22]. This product should have a reduced number of side-effects because it is a homologous immunoglobulin.

**PRODUCTION OF ANTITOXINS**

Producing an antitoxin is a time-consuming and expensive process. Host animals must be carefully maintained to avoid any contamination of the product with pathogens, the immunogens should be well characterised to ensure that the resultant product is sufficiently specific, the product may be enzymatically treated to improve its safety or efficacy, and the product must be purified to ensure the maximum specific activity and to minimise any side-effects. Antitoxins have proved to be very effective treatments for a range of intoxications, including snakebite [23] and overdosing with tri-cyclic antidepressants [24] and digoxin [25]. Much of the work carried out in optimizing these antibody-based therapies can be applied directly to the production of botulinum antitoxin.

**Choice of host**

Historically horses have been the most popular animals for the production of antitoxins because they are relatively easy to maintain and can produce large quantities of blood over a long period of time. The use of equine diphtheria antitoxin was responsible for the early advances against the disease, and all commercial botulinum antitoxins are currently produced in horses. Unfortunately equine antitoxins often show a very high rate of side-effects (serum sickness), which in extreme cases can lead to anaphylaxis. The protective ability of equine antitoxins is due to a subclass of IgG molecules, IgG T [26], which have a high immunogenicity due to glycosylation. IgGT has not been detected in preparations of other large animals such as goats and sheep, suggesting that these may be less immunogenic than equine IgG preparations.

Sheep are now being used preferentially for the production of antibodies for the treatment of digoxin poisoning. A comparison between ovine and equine antivenoms demonstrated that ovine antivenoms contained a higher percentage of venom-specific antibodies than the equine antivenoms, and provided better protection overall [27]. Results of extensive field trials have demonstrated an extremely low incidence (<1%) of side-effects with sheep anti-digoxin antibodies [28]. Raising antibodies in chickens, which produce IgY antibodies in response [29], may represent another way to reduce side-effects. As IgY cannot fix human complement, chicken antitoxins represent much safer antitoxins, and have the advantage that large amounts of antitoxin can be produced from eggs using the same conventional pharmaceutical methods that are used to prepare vaccines. It is important to remember that many animals may
contain communicable diseases which may compromise the safety of the product, and the recent issues regarding safety of bovine and ovine products and new variant Creutzfeld-Jacob disease need to be taken into account. Isolation of the host animals and careful screening and treatment of the product should allow the production of a safe antitoxin with little risk of communicable disease.

**Choice of immunogen**

The most common immunogen used for the production of botulinum antitoxin is formaldehyde-inactivated toxin (toxoid). This raises immediate problems, as antibodies that bind toxoid will not necessarily bind to native toxin and may not have any antitoxin activity. A solution to this is to immunise animals with toxoid for the first few immunisations, and once immunity to toxin has developed, boost with injections of toxin. The levels of toxin used to boost with can be increased once immunity develops, resulting in a high percentage of toxin-specific antibodies. Most antitoxins produced against botulinum toxin will have to be multi-valent, and there is a distinct advantage in using separate animals for each type of toxin. This prevents any problems caused by immunodominance of certain toxoids within a polyvalent immunogen, and allows for some toxoids being more immunogenic than others. Once the antitoxin activity of the serum from individual animals is known, serum can be blended to ensure a sufficient level of antitoxin to each type of toxin.

The Hc domain of botulinum toxin has been shown to be highly protective when used as a vaccine, suggesting that these fragments may represent good immunogens for antitoxin production. It is likely that the information obtained about the antibody-binding regions of botulinum toxin [30] will be used in future to design synthetic constructs to yield more active immunogens.

**Improving safety**

Heterologous antitoxins have a high incidence of side-effects, especially if the patient has been pretreated with serum for another condition. This often results in serum sickness or anaphylaxis, which in some cases can be fatal [31]. A Centers for Disease Control (CDC) study into the hypersensitivity reactions caused by botulinum antitoxins demonstrated that 9% of patients showed a hypersensitivity reaction to the antitoxin [32]. The incidence of serum sickness was linked directly to the amount of antitoxin received; patients who received more than 40 ml of antitoxin suffered significantly more serum sickness. All commercial types of botulinum antitoxin appeared to be identical in terms of side-effects.

Homologous antitoxins represent the most effective and safest form of antitoxin, show few side-effects, and have a long half-life, as they are not recognised as foreign protein. It is difficult to raise large amounts of antitoxins in humans and so this is an uncommon method, although there are some exceptions. Tetanus antitoxin is prepared from pooled plasma collected from blood donors, made possible as the majority of the population is immunised against tetanus and has a detectable antibody level to it [33]. Occasionally programmes are set up to produce small amounts of antitoxin by plasmaphoresing donors, such as the human botulism antitoxin program (hBIG [34]). Patients are immunized with the pentavalent botulinum toxoid produced by the CDC, which does not produce a high level of toxin-specific donor immunoglobulin, with the final titres being much lower than horse antitoxins [35]. Pharmacokinetic studies with hBIG have shown that administration of a 10 ml dose intravenously (containing 2500 IU of type A antitoxin) gave a serum titre > 1 IU/ml after 8 h, with all volunteers maintaining a protective titre (> 0.02 IU/ml) for at least 35 days [22].

Although not as safe as homologous antitoxins, despeciation should theoretically increase the safety of a heterologous antitoxin by removing the Fc region of the IgG molecules which binds complement and triggers inflammatory side-effects. However, use of an equine F(ab’)2 botulinum antitoxin demonstrated a 22% incidence of side-effects [36], suggesting that removing the Fc region may not reduce serum sickness per se, especially if the starting material has a relatively high immunogenicity. Studies in baboons and rabbits have demonstrated that the immune response to Fab fragments is far lower, and later than that to IgG [37]. Results obtained after clinical trials of anti-digoxin sheep Fab fragments demonstrated less than 1% side-effects in more than 700 patients [28]. Fab fragments are not associated with type III
hypersensitivity reactions, and do not bind complement or macrophages [37]. It has been possible to treat patients with enormous doses of Fab (in one case, 1280 mg was given to a 2-year-old child [25]) without any side-effects.

Another approach that could reduce the side-effects of antitoxins is to affinity purify the antitoxin using toxin. The fraction of toxin-specific antibodies in current botulinum antitoxins is often <2% of the total (C. Mayers, unpublished data). Affinity purification would reduce the amount of heterologous protein administered, and should greatly reduce the incidence and severity of serum sickness. Affinity purification of ovine antivenom increased its potency three-fold [38]. The major drawback to this process is the availability and expense of large quantities of toxin with which to affinity purify the antitoxins. There are also obvious safety concerns about producing large quantities of botulinum toxin for affinity purification of antibodies!

Improving potency

Methods such as boosting immunised animals with toxin rather than toxoid increases the potency of an antitoxin, as will any form of purification of toxin-specific antibodies. Fragmentation of an antitoxin to give a F(ab’)_2 or Fab product changes significantly the pharmacokinetics of the antitoxins or antivenoms [39]. This change in pharmacokinetics may have beneficial effects, as smaller fragments will have a larger volume of distribution and may be able to neutralise toxin that has left the circulatory system. Smaller fragments will also distribute much more rapidly than larger fragments, which may increase the therapeutic window for treatment when small fragments are used.

QUANTIFICATION OF ANTITOXIN POTENCY

An antitoxin can achieve its effect by a variety of possible mechanisms: blocking regions important for toxin uptake, blocking the catalytic site of the toxin, labelling the toxin for destruction, restricting the toxin to the circulation and enhancing the rate of clearance of the toxin have all been suggested as potential mechanisms. Any method for assaying antitoxin activity must take into account all of these factors, and must also account for the pharmacokinetics of toxin and antitoxin. The ideal antitoxin assay would measure the ability of an intravenously injected antitoxin to counteract a known dose of toxin given in a biologically relevant manner.

Current assays

The standard antitoxin assay currently required by the British Pharmacopoeia requires the initial establishment of a standard dose of toxin, known as the L+/-10 dose. The L+/-10 dose of toxin is defined as the smallest amount of toxin capable of killing a mouse when mixed with 1/10th of an international unit (0.1 IU) of a reference standard of botulinum antitoxin. Antitoxin is mixed with a graded series of toxin dilutions and injected intraperitoneally into mice. The mice are observed for 96 h and deaths are recorded. The lowest toxin dose required to kill the mice is regarded as the L+/-10 dose. As a guideline, an L+/-10 dose usually contains at least 1000 LD50 doses, although this can vary due to the activity of the toxin. Once this dose of toxin has been established the L+/-10 dose is mixed with test doses of the unknown antitoxin and injected into mice. The mixture that contains the maximum amount of antitoxin that still allows the mouse to die contains 0.1 U of the unknown antitoxin per dose. There are a number of physiological shortcomings with this assay: (i) toxin and antitoxin are pre-mixed before dosing. This would occur naturally in the blood, and at much lower concentrations than in a sample. These changes in solute and concentration may affect the binding of the toxin and antitoxin; (ii) toxin and antitoxin are administered by intraperitoneal injection. Toxin and antitoxin would normally meet in the blood, not the abdominal cavity. Although the toxin and antitoxin will be likely to drain into the blood via the lymphatic system there may be losses of both in the process due to absorption or adsorption in the body cavity; (iii) the assay relies on a lethal endpoint, with a correspondingly high severity level for the animals used. Any reduction in suffering to the experimental animals used would be welcomed by all involved.

This assay is currently the only method that will satisfy licensing authorities on the specific activity of botulinum antitoxins. It makes no assumptions as to the pharmacokinetic properties of the antitoxin, but makes the assumption that antibody–antitoxin binding is equal at any dilution.
(i.e. binding previous to injection into the animal is the same as the binding which would occur when the toxin and antitoxin are diluted inside the animal). The assay examines the ability to neutralise a high level of toxin; at lower levels of toxin (as the concentration approaches the dissociation constant of the antitoxin—toxin complex) proportionately more antitoxin will be needed. This was illustrated well by the results of Fulthorpe [40] who found that at levels of toxin 100-fold lower than the L+1/10 dose, 15 times more antiserum than predicted was needed to provide protection. This is due to the low avidity of the antitoxin. The avidity of each antitoxin will differ, and some will perform better at low concentrations of toxin.

Improved assays

Alternative methods for assaying toxin activity have been developed recently in a welcome drive to reduce the use of animals in experimentation. It is possible that these assays may be used to quantify antitoxin activity if suitably modified. Paralysis of the hind leg can be observed after a local injection of toxin, and can be used to measure toxin concentrations as low as 0.1 LD₅₀ doses [41]. Induction of abdominal ptosis after injection of the toxin into the abdominal muscle was suggested [42], and this has recently been refined and validated and found to be highly sensitive [43]. The effect of the toxin is scored by three independent observers on a graded scale with four levels of severity. This assay has proved to be far more sensitive and shows less variability than the LD₅₀ toxin assay, allowing toxin levels as low as 0.05 LD₅₀ doses to be measured. This assay could be used as an assay for monitoring the efficacy of antitoxins, as long as a standard toxin preparation was available to test the unknown toxin against, and would be highly preferable to the lethal assay in terms of accuracy and animal welfare. However, the results obtained so far have only been from IgG or F(ab')₂ antitoxins, which are likely to have similar pharmacokinetics (i.e. similar dispersal from the site of local botulism). Antitoxins produced from very small fragments (Fv or Fab) may diffuse from the site very quickly, and their efficacy may be underestimated by this non-lethal assay. The non-lethal assay needs to be evaluated with a range of types of antitoxin, although it appears to have a great deal of potential.

The ideal antitoxin assay would mimic the natural course of events following botulimum intoxication. It is not clear how well the results obtained from local botulism can be extrapolated to systemic botulism. An alternative botulimum antitoxin assay that may mimic both natural, food-borne botulism as well as inhalation botulism could be suggested: (i) dose mice orally with toxin, or inject a standardised quantity of toxin intraperitoneally into the mouse. The uptake of toxin injected intraperitoneally may be similar to the uptake from the gut or lung, whereas intravenous injection is likely to give an immediate and complete distribution of toxin. It would prove extremely informative to assay an antitoxin’s potency at varying levels of toxin, spanning the range seen in physiologically relevant intoxication rather than at a single high dose; (ii) inject a fixed amount of antitoxin intravenously via the tail vein. It is likely that prophylactic use of antitoxin will be by intravenous injection. The length of time between injecting the toxin and antitoxin could be varied, in order to gain some insight into the therapeutic window; (iii) monitor mice by sensitive tests such as those used in the non-lethal assay described above. Score mice, and terminate experiment before severe signs of botulimum intoxication are seen.

This assay accounts for all the factors involved in clinical botulism, and is a true measure of the ability of an antitoxin to inhibit systemic, rather than local botulism. The non-lethal assay [43] may prove equally reliable, although it assumes that all antitoxins will display similar pharmacokinetics – a dangerous assumption.

ASPECTS OF THERAPY

Dosing levels

In order to know how much antitoxin should be given to a patient to counteract the effects of impending botulimum intoxication after an exposure, two factors need to be ascertained. Firstly, how much toxin has the patient been exposed to, and secondly, what is the specific activity of the antitoxin to be used.

Monkeys and mice were both found to have similar blood concentrations of toxin after death when given the same dose of toxin in LD₅₀ (i.e. 5 mouse LD₅₀ produces the same blood toxin
concentration in a mouse as 5 monkey LD$_{50}$ produces in a monkey [44]). This suggests that the lethal dose (in terms of blood toxin concentration) for mouse and monkey would be approximately the same. Monkeys and mice are said to be equally sensitive to type A toxin [1] on a per kg basis. The same is true for the other types of botulinum toxin with the exception of type D, to which monkeys are 100-fold less sensitive on a per kg basis [1]. This allows the human parenteral lethal dose to be estimated at 1 ng/kg for type A, B, C, E, F and G and 100 ng/kg for type D. The maximum level of botulinum toxin detected by the CDC in human serum is 32 mouse LD$_{50}$/ml [45], with the highest level ever detected being 160 mouse LD$_{50}$/ml of type E toxin [46]. Assuming that the toxin is equally distributed throughout the extracellular fluid (approximately 15 litres in an average adult), this would give a total of 2 400 000 mouse LD$_{50}$ of toxin in the patient with the highest ever detected blood toxin level. It is important to remember that this represents the highest serum toxin concentration ever observed, and lower concentrations are more likely. This can be taken as a rough guide as to the maximum amount of toxin likely to be in a patient after food-borne intoxication. It is impossible to say what sort of levels would be seen in a patient exposed in a biowarfare incident. More accurate predictions could be made given the pharmacokinetic properties of the toxin, about which very little is known.

If the maximum level of toxin in a human is likely to be around 2 400 000 mouse LD$_{50}$, how much antitoxin would be required to neutralise this amount? One-tenth of an IU of antitoxin should neutralise at least 1000 mouse LD$_{50}$ of toxin (by definition), so to neutralise 2 400 000 mouse LD$_{50}$, 240 IU of antitoxin would be needed. Dosing with approximately 9000 IU of antitoxin gives a maximum plasma concentration of 5 IU/ml [45], suggesting a volume of distribution of approximately 25 ml/kg body weight; 240 IU of antitoxin would be expected to give a final concentration of 0.13 IU/ml antitoxin. Human botulinum antitoxin has also been shown to be highly protective in monkeys against an aerosol challenge with antitoxin levels well below this level (protective levels of 0.025 IU/ml [47]).

The current Connaught botulinum antitoxin is required to contain 7500 IU of type A botulinum antitoxin, although the vials contain an extra amount of bulk ABE antitoxin to give a 20% excess, giving a final dose of around 9000 IU/vial. Dosing a patient with 9000 IU to get a serum level of 5 IU/ml represents approximately 40 times the level of antitoxin needed (calculated above) to eliminate the maximum recorded human dose of toxin. Some patients have been dosed with more than 40 ml (four vials) of antitoxin [32] containing at least 30 000 IU of type A antitoxin, which amounts to approximately 125 times the calculated required level. Although a margin of error is clearly desirable when dosing a patient with antitoxin, this level of dosing seems excessive, and will increase the risk of side-effects significantly. There have been repeated suggestions to reduce the dose of antitoxin given to a more realistic level [34,45,48].

**Therapeutic window**

An important aspect of botulinum antitoxin therapy is the therapeutic window – the time period in which treatment with an antibody will have a therapeutic effect. Index patients obviously only receive antitoxin on showing symptoms, whereas related cases usually receive antitoxin much more quickly after exposure to toxin. Injection of human antitoxin could protect mice 2 h after treatment with toxin but could not protect against severe intoxication 10 h after toxin treatment, no matter how high the antitoxin dose [49]. Similarly, equine F(ab')$_2$ antitoxin was shown to be 100% effective in protecting guinea pigs from a lethal challenge, but decreased to 10% effectiveness when treatment was delayed until 24 h after treatment [48]. A retrospective study demonstrated that patients who received antitoxin within 24 h of exposure to the toxin had a shorter course than those who received antitoxin later [21]. It is therefore extremely important that antitoxin be administered as soon as botulinum intoxication is suspected. There is still justification in using antitoxin once symptoms have developed: 80% of monkeys given 2.5–5.0 LD$_{50}$ of toxin could be saved by the use of antitoxin immediately after they became symptomatic as long as suitable nursing care (feeding and antibiotic use) was provided [50]. The reluctance to administer antitoxin immediately is due to the high incidence of side-effects, and improvements in the safety of antitoxins should improve this matter considerably. The development of a fast and
highly sensitive system for detecting toxin in serum would allow faster diagnosis, and would allow for earlier dosing with antitoxin.

CONCLUSIONS

Although the current botulinum antitoxin is moderately effective, many improvements could be made to increase its safety and potency. These modifications would result in an extremely safe, effective antitoxin, removing any hesitance to treat a patient once botulism is suspected. It may be possible to increase the potency of antitoxins (and also to increase the therapeutic window) by choosing antibody fragments with a large volume of distribution. This should also reduce the side-effects caused by treatment to an acceptable level. Measurement of the pharmacokinetic properties of botulinum antitoxin and toxin would also provide supportive data allowing for the selection of the optimal antitoxin. The future of antibody based therapies appears bright – recent advances in humanised monoclonal antibodies and recombinant antibody products should eventually replace the use of animals in producing antitoxins, resulting in a safe, well characterised product produced at a much reduced cost.

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