1 Introduction

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1.1 Introduction

This book has been written to highlight the remarkable progress in the application of botulinum toxin in medical practice. It is used across many specialties and has an increasing indication across a whole spectrum of diseases. As a result, its commercial sales have grown exponentially and its use in cosmesis has made ‘BOTOX\textsuperscript{TM}’ a household name. This is extraordinary after such a short time in this field and some other products have even gone so far as to add an ‘-ox’ on the end of their brand name to attempt to capture some of the kudos (and market) of botulinum toxin. This is of course very different from when the drug was first marketed and when it was regarded as a highly dangerous product. The indications for botulinum toxin treatment are listed in Chapter 5. In many, there is still little or no evidence that it works, but in others, there is good evidence of its therapeutic benefit.

1.1.1 History of BoNT

Botulinum toxin was first identified as a poison in the nineteenth century. The toxin is a protein, which is produced by the Gram negative \textit{Clostridium botulinum} bacterium. It is found in a variety of foods, but is most common in meat products. The name botulus means sausage and hence its terminology from its appearance in meat products. The features of botulism have been known since around the time of Christ and it was certainly described in the Middle Ages. However, it was not until 1817 that the German physician Justinus Kerner wrote on the role of an infective agent in food-borne poisoning\textsuperscript{1}. He then published a monograph on poisoning in 1820 in which he described the features, made many original observations and commented on the possible causation, diagnosis and treatment\textsuperscript{2}. He concluded that a toxin produced by an infective agent was responsible for the features of paralysis of skeletal and smooth muscles. He published...

a second monograph in 1822, in which he laid out his hypotheses on BoNT and described clinical evaluation of the problem through case histories of his patients and through post-mortem examination of patients with botulism. He even ventured to infect himself and noted that while motor signals were always involved, sensation was always preserved. Most remarkably, he proposed that this toxin could be turned into a therapeutic agent for the control of chorea and other neurological diseases. It was left to the German physician, Muller, to coin the description, botulism, to connect the infection to meat products and, in particular, the sausage.

Pierre van Ermengem was Professor of Microbiology at the University of Ghent, Belgium, when he was asked to investigate an outbreak of botulism in the nearby village of Ellezelles. The illness appeared to follow a funeral ceremony and he was able to isolate the anaerobic bacterium *Bacillus botulinus* from the food and from the victims. This was not the end of the story since there had been several outbreaks which confirmed the incriminating cause. Van Ermengem himself experimented on small animals and observed the typical features when they were fed with infected meat products. The isolated bacteria were the same anaerobic bacilli as before. Fortunately, efforts to control infection have been successful through better public health and meat hygiene standards. The bacterium was renamed in the twentieth century to *Clostridium botulinum*.

The potential for using the toxin in the military arena was also quickly realized and the USA led the way. The laboratory at the military camp, Fort Detrick, was used as a place to judge the effects of the toxin and much of our early knowledge of the agent comes from the studies carried out there. This followed work from Dr Hermann Sommer in the 1920s, which paved the way for identifying the toxin subtypes. He attempted to purify BoNT type A. Immunization against infection for workers at Fort Detrick was the first attempt to control the toxin. A toxoid was used as a vaccine.

### 1.1.2 History of BoNT as a therapeutic agent

The story starts in 1946 when Dr Carl Lamanna first crystallized BoNT type A. He described the toxin’s components, but did not identify them as heavy and light chains. Fort Detrick produced purified toxin for other research groups, from which came a great deal of knowledge on the agent’s structure, action and characteristics. From this basic knowledge, came the crucial result of a British study group that botulinum toxin blocked the release of acetylcholine from synaptic vesicles at neuromuscular junctions. It was thereafter a short step to deduce that the toxin may be able to block these junctions therapeutically in overactive muscles. The role of acetylcholine was described in neuromuscular control...
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and in its responsibility for trophic activity in muscles. Acetylcholine blockade by botulinum toxin resulted in muscle atrophy by paralysing the muscle. Ophthalmological experiments started in the early 1970s, when Dr Alan Scott, an American ophthalmologist, experimented with several drugs in trying to find a suitable alternative to surgery for childhood strabismus. Botulinum toxin had immediate advantages through its relatively long action and he documented its promising role in 1973. He also looked at other toxins during this period, but felt that BoNT had promise. Clinical studies in humans did not take place until 1977 when he injected a patient with strabismus. In his trial of 1982, he and his colleagues were able to observe that it was well tolerated and reversible and was also effective in nystagmus, blepharospasm, hemifacial spasm and even spasticity. Its activity will be described in Chapter 2 where reference will be made to early clinical studies.

The commercial preparations have received individual attention for registration purposes, as their characteristics are not identical. Both BOTOX® and Dysport® are licensed for the treatment of blepharospasm, hemifacial spasm and cervical dystonia and for the treatment of spastic lower leg problems in children with cerebral palsy over the age of two years. BOTOX® is now also licensed for upper limb post-stroke spasticity. Botulinum toxin type B (Myobloc (USA) or Neurobloc) entered the commercial arena in the late 1990s and received a licence for cervical dystonia in 2001 in the USA and many European countries. Recently, Merz, a German company, has released Xeomin which is a type A botulinum toxin free of complexing proteins (see Chapter 5).

Thereafter, its history has been well documented and has started to be used in patients with facial tics, blepharospasm, cervical dystonia and hemifacial spasm, and now many other indications — the subject of this book.

1.2 Why write this book?

Since this first usage there has been a steady increase in the number of clinical indications. There are now in excess of 100 reported uses of botulinum toxin in the world literature. Whilst many of these uses are still in the form of individual case reports and open studies there are nevertheless a significant number of indications that now have a robust and sound evidence base. Despite the dramatic increase in world literature on the clinical use of botulinum toxin there are surprisingly few textbooks that have tried to amalgamate the evidence into a single practical textbook — hence this volume.

We have several aims in writing this book. First we wanted leading clinicians in their field to write clear, practical chapters on their particular area of expertise.
The aim is for each author to provide a résumé and critique of the evidence base for the use of botulinum toxin in that particular clinical context. We have also asked each author to place the use of botulinum toxin in the context of other available treatment possibilities. In many conditions, such as spasticity, botulinum toxin is not a treatment in isolation but is simply part of the overall package of care for the individual patient. We also wanted each chapter to be clear and readable and offer a straightforward practical guide in the use of botulinum toxin. However, each chapter should not give the impression that there is a standard protocol for each individual use of botulinum toxin. This is certainly not the case. Each chapter should give a balanced review of the literature so that the individual clinicians can determine whether botulinum toxin is suitable and, if so, gain some idea of not only the usefulness of the technique but also the practicalities of the injection for each indication. Each patient is very different and thus each will need a personalized approach to the use of botulinum toxin for their particular problem.

1.3 Outline of the book

We have divided this book into two sections. The story of the clinical development of botulinum toxin is fascinating. This class of neurotoxin offers great future potential for the development of other therapeutic products. We feel that clinicians should have at least a basic understanding of the underlying chemistry and mode of action of the product, so one of the world’s leading figures in the development of botulinum toxin — Professor Oliver Dolly — was asked to introduce the neurochemistry and mode of action of the toxin in Chapter 2. The commercial development of botulinum toxin is one of the success stories of recent years in terms of basic science being translated into practical clinical use in a relatively short period of time. Much of the early commercial development of botulinum toxin was at the Centre for Applied Microbiology and Research (CAMR) at Porton Down in the UK. CAMR developed a stable freeze-dried type A toxin formulation initially supplied to Moorfield’s Eye Hospital in London. However, as the use of the toxin grew rapidly, CAMR developed the product commercially with Porton Products (now Ipsen Ltd) which eventually led to the approval by the Medicines Control Agency for licensed usage of strabismus, blepharospasm and hemifacial spasm in 1990. The product was also being developed commercially at the same time in the USA by Oculinum Inc. which was acquired in 1998 by Allergan. Ipsen now market the formulation, now called Dysport and Allergan market the formulation called BOTOX®. The story of the commercial development is outlined by Peter Hambleton, Andrew Pickett and
Clifford Shone in Chapter 3. This chapter also discusses the therapeutic potential of the botulinum neurotoxins as efficient neuronal delivery vectors as well as development of the neurotoxins as therapeutic analgesic agents.

The second section of the textbook brings together the evidence of the clinical efficacy of botulinum toxin for different indications. However, the principles of injection are generally similar and the practical issues of injection techniques are given by Tony Ward in Chapter 5. This chapter also outlines the side-effect profile as well as the practical differences between the different botulinum serotypes. The latter is a particularly important point as BOTOX® units are not the same as Dysport units. It is essential that clinicians understand that the two products do not have the same unit base. The situation is further complicated by the emergence of botulinum serotype B (Myobloc or Neurobloc — manufactured by Elan) and more recently Xeomin (manufactured by Merz) which is type A toxin free of complexing proteins. Clinicians new to the use of botulinum toxin should read this chapter prior to subsequent chapters on individual indications.

Although strabismus was the first clinical usage of botulinum toxin in man the product largely developed as a treatment of first choice for dystonia. Khalid Anwar maps the development of botulinum toxin for the management of cervical dystonia and discusses the various approaches to treatment of this disabling condition. Use of botulinum toxin for the management of focal dystonia has dramatically changed the lives of people with this condition. Prior to the advent of botulinum toxin there was very little that could be effectively done to manage dystonia but with success rates in excess of 90 per cent the condition can now usually be controlled and many individuals can go on to lead normal lives.

Although cervical dystonia is the commonest form of focal dystonia there are other rarer, but equally disabling types. Maurice Hawthorne and Khalid Anwar write about oromandibular and other laryngeal dystonias and dysphonias and they provide a good summary of the techniques behind these rare but important conditions (Chapter 7).

Although the management of dystonia has been revolutionized by botulinum toxin it is probably the use of botulinum toxin for the management of spasticity that has had most influence. There is a now a robust evidence base confirming the efficacy and safety of botulinum in the management of focal spasticity following stroke, traumatic brain injury, multiple sclerosis, cerebral palsy and a variety of other spastic conditions — in both adults and children. Tony Ward (Chapter 8) reviews the evidence and offers practical suggestions for the usage of botulinum toxin for these common problems. The chapter emphasizes that although botulinum has made a big impact it is not a treatment in its own right and needs to be...
combined with a range of other antispastic measures such as appropriate seating, physiotherapy, orthosis and the judicious use of oral medication. Botulinum toxin is an invaluable addition to our therapeutic armoury in the overall management of spasticity.

Although botulinum is mainly known for its muscle relaxation properties it also has profound and useful effects on the autonomic nervous system. It can have an extraordinary effect on hyperhidrosis (Chapter 9) as well as hypersalivation (Chapter 10). Such conditions can be extremely socially embarrassing and there are regrettably few practical alternative treatments. For example, many children with cerebral palsy have been socially isolated by constant dribbling. This is equally a source of embarrassment and isolation in the adult population with, for example, Parkinson’s disease or motor neuron disease. Individuals with excessive sweating can also suffer major embarrassment and social and economic isolation as a result of their condition. Botulinum toxin can produce exceptional results for such people.

Botulinum toxin is also an analgesic agent and indeed Chapter 3 highlights the potential role of botulinum toxin in the management of chronic pain. The current usage in headaches (Chapter 11) and back and neck pain (Chapter 12) are discussed by David Dodick and Aine Carroll. The evidence of existing serotypes of botulinum toxin as an effective analgesic agent is not yet strong but nevertheless a number of good quality studies confirming the analgesic efficacy are now emerging. Botulinum is not likely to be a panacea for chronic pain but will clearly have a place, particularly for the various pain syndromes characterized by muscle spasm. Hopefully we will hear more in the future as botulinum toxin is further developed as an inhibitor of neurotransmission from pain conducting neurons.

One of the pioneers of the clinical use of botulinum toxin was John Elston when he was working in the early days of development at Moorfields Eye Hospital. He has written a wonderful chapter on the more established as well as the newer indications in clinical ophthalmology. Whilst botulinum still has a role to play in the management of strabismus it also has an important and thus a key role in the management of blepharospasm, hemifacial spasm and apraxia of eye opening as well as rarer indications such as post-facial palsy problems and the therapeutic induction of ptosis.

A fascinating and newer development has been the use of botulinum toxin for the management of bladder and bowel difficulties. This particularly includes the management of detrusor overactivity and the management of anal fissure. The practicalities and the place of botulinum in the management of these and other bladder and bowel problems are outlined in Chapter 14 by Giuseppe Brisinda.
Botulinum toxin continually features in the lay press. Such exposure is often useful as we have found in our clinics that after every press article there is a spate of new, and often appropriate, referrals for injection. However, the lay press have mainly focused on the use of botulinum toxin from the management of wrinkle lines and other cosmetic usages. There is little doubt that the practice of cosmetic surgery has been advanced by the safe and effective treatment of wrinkle lines with botulinum toxin. The techniques and results are demonstrated in Chapter 15.

Finally, there are a number of chronic neurological problems characterized by various degrees of muscle spasm. Many of these conditions are not particularly amenable to any intervention and botulinum toxin has been tried with some success in these conditions even though it is unlikely that such indications will ever be part of the therapeutic licence. These conditions include tics, myoclonus, stiff person syndrome, Parkinson’s disease and tremor as well as the rarer forms of limb dystonia such as writer’s cramp and other occupational cramps. These conditions are discussed by Mike Barnes in Chapter 16.

Overall, this book is not intended as a totally comprehensive review of all indications ever published for the use of botulinum neurotoxins. However, we hope that we have covered all the standard indications as well as reviewing the more important and the more promising indications that already provide further practical use of botulinum toxin and may do so increasingly in the future. The story of botulinum toxin has been a significant story of success and translation of basic neuroscience to practical reality. The story is not yet completed and there is every hope that botulinum neurotoxins will provide a platform for exciting clinical developments in the future. However, we hope that this textbook has provided the clinician, and the basic scientist, with a practical and readable guide to the development of this fascinating clinical entity.

REFERENCES


Mechanistic basis for the therapeutic effectiveness of botulinum toxin A on over-active cholinergic nerves

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2.1 Introduction

Seven homologous variants (serotypes A–G) of botulinum neurotoxin (BoNT) are produced by different Clostridium botulinum, and closely-related toxins have been isolated from C. butyricum and C. barati. All are proteins with $M_r \approx 150$ K which are activated by selective proteolytic cleavage to yield a heavy chain (HC) and a light chain (LC) linked by a disulphide bond and non-covalent interactions. Each exhibits amazingly high specific neurotoxicities ($10^7$–$10^8$ mouse LD50 units/mg) after separation from their naturally-occurring complexes with accessory proteins. The size and composition of such complexes differ for each serotype; for example, type A can be isolated as large assemblies (LL or L forms with $M_r \approx 900$ or 450 K) of the active moiety, BoNT, with non-toxic non-haemagglutinin and several haemagglutinin proteins.

Long before the recent spiralling interest worldwide in type A toxin as a therapeutic for weakening hyper-active muscles, BoNTs had been adopted as informative probes for delineating the fundamental process of quantal transmitter release. This choice was based on their renowned abilities to induce neuromuscular paralysis by presynaptic inhibition of acetylcholine (ACh) release, with exquisite specificity (i.e. without affecting any other measured parameters such as ion channels in the nerve terminal, ACh synthesis, etc.). Also, other toxins had been shown to be useful for the biochemical characterization of neurotransmitter receptors and cation channels. Another attraction of using BoNTs was that motor nerves in frog paralysed with type D did not atrophy or undergo any detectable ultrastructural changes over ~50 days; likewise, mammalian nerve endings treated with type A did not degenerate but, instead, underwent remodelling that culminated in full recovery of neuro-exocytosis after 90 days.
2.2 Outline

In view of such unique potency and selective perturbation of a key step in synaptic transmission, which was very poorly understood in molecular terms despite Nobel prize winning contributions\textsuperscript{12}, it was prudent to expect that utilization of BoNTs as research tools would unveil a functionally important component. Nonetheless, some eminent scientists doubted the wisdom of working with such exotic and dangerous toxins. However, the commitment to this experimental approach yielded an even larger measure of success than needed to compensate for the difficulties in safely handling such potent neuroparalytic agents. Notable outcomes, to be reviewed herein in chronological order, include establishing molecular/mechanistic bases or evidence for the following:

(a) the targeting of BoNT/A and /B to cholinergic nerve endings in the periphery by binding to distinct ecto-acceptors located exclusively thereon, a step requiring the correct conformation of the HC;

(b) internalization of the toxic moiety by acceptor-mediated endocytosis and translocation to the cytosol, a process enhanced by neural stimulation;

(c) intracellular inactivation of ubiquitous target(s) essential for the release of all transmitters from small synaptic vesicles and large dense-core granules;

(d) light chain of type A inhibits ACh release at the neuromuscular junction, due to its reported Zn\textsuperscript{2+}-dependent endoprotease cleaving and disabling a SNARE protein, SNAP-25, which together with synaptobrevin and syntaxin — the substrates for BoNT/B/D/F/G and /C\textsubscript{1} (reviewed in\textsuperscript{13,14}) — were, thus, found to be responsible for Ca\textsuperscript{2+}-regulated exocytosis; and

(e) pinpointing factors contributing to the prolonged, but reversible, neuromuscular paralysis caused by BoNT/A including the persistence of its cleaved target, longevity of the protease activity and extended time course of the nerve sprouting/remodelling induced. Such an unique combination of remarkable multi-functional activities underpins the impressive success of type A toxin as a first-choice therapeutic for an ever-increasing number of conditions arising from over-activity of cholinergically-innervated muscles.

2.3 BoNT/A binds to ‘productive’ ecto-acceptors on motor and autonomic nerves: such targeting underlies its peripheral cholinergic specificity

Electrophysiological recordings in rodent phrenic nerve diaphragm\textsuperscript{15} revealed that type A BoNT reduces the frequency of miniature end-plate potentials and blocks neurally-evoked end-plate potentials, without altering their