Toxins to drugs: The case of botulinum toxin

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Botulinum toxin has been touted as the most fatal of all poisons. And today it is being used for diminishing the visible signs of ageing. But besides cosmetic uses, the deadly toxin has several therapeutic uses. Botox has received a lot of publicity in the US and now in India too. More and more questions are being raised regarding its use.

Botox or botulinum toxin (BTX) is the latest revolution in the field of rejuvenation products. Treatment with Botox is said to take years off one's face by easing out wrinkles. In a recent paper by Lim and Raymond[1], other medical uses of the toxin are listed. This was adjudged as the best paper and was awarded the 2007 Horrobin Prize for medical theory, in honour of David Horrobin, the renowned researcher, biotechnology expert and founder of the journal *Medical Hypotheses*, who died in 2003. According to the judge V. S. Ramachandran, an internationally known neuroscientist, University of California, San Diego, USA, the paper remains a scholarly overview for valuable new directions of research.

The researchers used BTX developed from exotoxin of *Clostridium botulinum* and carried out studies to show that the toxin causes chemo-denervation of cholinergic neurons in the brain. Clinically, uses of BTX have increased now and it can be used to treat overactive skeletal and smooth muscles, excessive secretary and painful disorders.

Researchers have mentioned several uses of BTX, which include treating excess secretions from the glands, eye-squint and muscle spasm disorders. The toxin can also be used for easing restless leg syndrome, reducing perspiration, improving breathing in asthma and removal of excess fat.

With so many uses of Botox, it is difficult to digest the fact that BTX is one of the most poisonous substances which occurs naturally. As the name suggests, it is a highly toxic neurotoxin; a protein released by the bacterium *C. botulinum*. Merely 0.0000007 g can kill adults. In the 1940s, BTX was developed into a biological weapon by the US and other countries.

The toxin is a deadly poison that can cause botulism, a rare but serious paralytic illness[2]. Botulism causes nerve paralysis and respiratory failure. There are three types of botulism; first, food-borne botulism in which bacteria produce the toxin in the presence of little oxygen as in canned foods; second is wound botulism in which the bacteria can get inside the wound-producing toxin, and lastly infant botulism[3], in which the bacteria grow inside a baby's intestinal tract between the ages of 6 weeks and 6 months. Wound botulism is associated with intravenous drug use leading to paralysis of motor and autonomic nerves causing respiratory failure[2]. It is more common in people who inject heroin, which contains bacterial spores. Infant botulism can come from honey, corn syrup or exposure to soil contaminated with the bacteria. Injection of antitoxin helps in food-borne and wound botulism. Antitoxin does not help in case of infant botulism. In this case botulism immune globulin is used for treatment.

Although the major source of the toxin is *C. botulinum*, *C. barati* and *C. butyricum* also produce the toxin[4]. There are seven serotypes of this toxin – A, B, C, D, E, F and G. Among these, serotype A, i.e. botulinum toxin type A is the one which is approved by the Food and Drug Administration (FDA, USA) for medical treatment.

The Botox story

It was in the 1800s that a German poet and physician, Justinus Kerner identified an anaerobic bacterium *C. botulinum* as the cause of food-poisoning[5]. The bacterium grew in badly handled or processed meat products and caused poisoning, resulting in gastrointestinal problems. The disease then was called botulism by another German physician John Muller in 1870. The name botulism comes from Latin *botulus* meaning sausage. The name was apt because the fatal food-poisoning outbreak in Stuttgart in Germany at that time was caused by eating partially cooked sausages. And Kerner was the one who identified the clinical symptoms. After experimenting with the extracts from sausages, he postulated that the extracted toxin can be used to block the hyperactivity of the sympathetic nervous system.

In 1895, Pierre Emile van Ermengem, a Belgian professor of bacteriology isolated the bacterium *Clostridium*, which was then cultured by Edward Schantz, a young Army Officer stationed at Fort Deitrich, Maryland, USA, in 1944 to extract the purified toxin in crystalline form. In 1949, Burgen and his colleagues reported their findings[6] stating that BTX blocks neuromuscular transmission. And this was confirmed by Alan B. Scott, an ophthalmologist at the Smith–Kettlewell Eye Research Foundation, San Francisco in 1973, in animal experiments in non-human primates, where he discovered that botulinum A causes muscle paralysis. After eight long years, Scott was successful in treating strabismus-squint; muscle spasms around the eyes in humans. This laid the ground for the potential therapeutic uses of botulinum A in the treatment of neurological and muscular disorders – overactive skeletal and smooth muscles, migraine, trigeminal neuralgia, etc.[7–9].

In 1978, the FDA approved the testing of botulinum toxin type A in human volunteers for these conditions. The initial toxin treatment was developed and marketed under the name 'Oculinum' by Scott, with support from the National Eye Institute, National Institutes of Health, USA.

In 1987, Canadian ophthalmologist Jean Carruthers and her husband, British-born dermatologist Alastair Carruthers, University of British Columbia, USA, accidentally discovered that when they used the toxin to treat eye muscle spasm, the crow's-feet lines around the eyes improved. The couple went on to become Botox pioneers. And this was the beginning of the use of Botox for cosmetic correction.

Mechanism of action

Botulinum toxin A, a zinc proteinase, blocks the activity of the cholinergic

nerves that control the muscles and glands[1]. When Botox is injected into the muscle, in the first phase the nerve–muscle action is blocked by preventing the release of the neurotransmitter acetylcholine, thus preventing muscle contraction. This is basically the local effect of the toxin.

The process comprises binding of the active ingredient in the toxin to the cell membrane of the motor nerve to begin targeted treatment at the injection site of the toxin. Following binding there is internalization into the cytoplasm of the motor nerve cell membrane via endocytosis. It is here that the toxin protein molecule is activated. The activated protein molecule then cleaves with a particular protein at varied locations, thus blocking the nerves from releasing acetylcholine[10]. The result is blocked muscle contractions with reduced muscle activity. In addition, the release of neuropeptides involved in the transmission of painful sensations is also blocked.

The second phase of action is when the nerve–muscle communication is restored. The effect of BTX is therefore temporary. The nerve impulse activity and muscle contractions resume within a course of time. This happens when new nerve endings sprout and the original nerve-connection is re-established.

Preparation of BTX for clinical use

In its concentrated form, BTX can cause botulism. However, researchers have proved the effectiveness of the toxin in patients with muscle and nerve disorders[11]. Botox as the toxin is commercially known, is a purified protein; a diluted form of BTX. It is made by fermentation of the Hall strain of *C. botulinum* type A organism[12]. It is sterilized and vacuum-dried in the laboratory. The vaccine that is injected routinely is known as Botox type A vaccine and is commercially available in crystalline form packed in vials or bottles in 100 units. Botox is refrigerated and stored at temperatures of 2–8°C and is diluted with saline solution prior to injection in the muscle through fine hypodermic needles.

It was in April 2002 that the FDA approved the cosmetic use of Botox. And it was in 2004 that FDA approved the use of Botox as a treatment for excessive underarm sweating or hyperhidrosis. In India, BTX protein is sold under the brand name 'Botox'. Botox is manufactured by Allergan Inc., an American company for therapeutic as well as cosmetic purposes. Allergan acquired the rights of 'Oculinum' in 1989 and went on to experiment with Botox thereafter. The other trade names of Botox include 'Dysport' and 'Vistabel'. Myobloc B/Neurobloc, a purified protein from a different form of BTX comes in a liquid form and is refrigerated until use. The protein is similar in effectiveness to Botox.

Therapeutic uses of Botox

Interestingly, BTX is the first biological toxin which is licensed for the treatment of human diseases. Botox is best known for its use as a wrinkle remover in the frown lines between the eyebrows and crow's-feet. When injected, it restricts the muscle, movement by paralysing the muscle, thus removing wrinkles and imparting a smooth appearance to the skin. This effect, however, lasts for about 3–6 months. Under normal circumstances, once the Botox effect has worn off, the same lines and wrinkles will return on the skin[12].

Several therapeutic uses of Botox have been cited in the literature[13–15]. In general, BTX can help treat pain, eye squint, excess gland secretion and muscle spasm disorders. Botox is approved by the FDA for the treatment of blepharospasm (spasm of the muscles of eyelids), cervical dystonia (a movement disorder with severe neck muscle spasms) and hyperhydrosis (excess sweating). Myobloc is approved for the treatment of cervical dystonia, which occurs in adults and it is used mainly for children who do not respond to Botox injections.

Botox is also used for spasticity, i.e. involuntary muscle tightness and stiffness, which is seen in individuals with stroke, cerebral palsy, multiple sclerosis and those with severe head injuries. The protein is directly injected into a spastic or dystonic muscle to reduce the tightness of the muscle by weakening it. Generally, injections are given in different areas of the muscles and 4–6 injections may be required at a time. A topical anaesthetic may be used to numb the skin.

Botox injections in men with benign prostatic hyperplasia (enlarged prostate gland) eased symptoms and improved quality of life up till a year following the procedure[16,17]. Botox is also used in the treatment of severe tension headache, migraine, overactivity of oesophageal and stomach muscles and glands, neurological bladder dysfunction, trigeminal neuralgia (stabbing facial pain), back pain and tennis elbow (inflammation of the elbow). The other possible uses of Botox are improving breathing in asthmatic patients, calming restless leg syndrome and removal of excess fat – chemical liposuction.

Adverse reactions

Treatment with Botox can be expensive. Since it is a non-surgical treatment, it is popular, although with some trepidation regarding its use. The common side effects of Botox injection include headache, especially when the injection is given in the forehead. Flu-like respiratory symptoms, nausea, rash, itching, etc. are common too. Evidence so far indicates that no long-term side effects directly linked to the use of Botox have been seen. However, FDA announced in February 2008 that it is currently reviewing the safety data for BTX preparations.

The labels on the available products flash a list of adverse reactions or side effects that can occur following injections. These include dysphagia (difficulty in swallowing), difficulty in speaking, cervical dystonia, which causes involuntary contracting of the neck muscles, causing abnormal movements and awkward posture of the head and neck, or ptosis (drooping of eyelids). There are chances of muscle weakness and paralysis of muscles.

Botox use has been advised against certain medical conditions and in certain patients[12]. Those individuals having skin infections, for instance, eczema or psoriasis, or those taking amino glycosides and antibiotics, which affect the kidney function, should not use Botox. Also, people suffering from myasthenia gravis, muscular dystrophy, double vision and Parkinson's disease should avoid using Botox.

The toxin is not advisable for those who are emotionally disturbed. For people over the age of 65 years, the response of the toxin is slow and hence there can be unpredictable effects. The dose may need to be adjusted, if at all it is used. Botox is occasionally known to cause an allergic reaction due to the protein content of the vaccine. The vaccine can be used only after performing an allergy test.

Although there are not many studies regarding the use of Botox in pregnant women, experts advice against its use during pregnancy, in nursing mothers and even in women who are planning a pregnancy. Botox use under the age of 18 years is generally avoided.

Theoretically, BTX can be used to treat pain, skeletal and smooth muscles and glands – all of which receive a cholinergic nerve supply. And the likelihood of treating arrythmias or cardiomyopathy or tumours using the toxin is not far-fetched. Yet the pros and cons have to be weighed before Botox can be called a miracle drug.

1. Lim, E. C. H. and Seet, R. C. S., *Med. Hypotheses*, 2007, **69**, 718–723.
2. Wenham, T. N., *Emerg. Med. J.*, 2008, **25**, 55–56.
3. Tseng-Ong, L. and Mitchell, W. G., *J. Child Neurol.*, 2007, **12**, 1333–1337.
4. Montecucco, C. and Schiavo, G., *Mol. Microbiol.*, 1994, **13**, 1–8.
5. Erbguth, F. J. and Naumann, M., *Neurology*, 1999, **53**, 1850–1853.
6. Burgen, A. S. V., Dickens, F. and Zatman, L. J., *J. Physiol.*, 1949, **109**.
7. Piovesan, E. J., Teive, H. G., Kowacs, P. A., Della Coletta, M. V., Werneck, L. C. and Silberstein, S. D., *Neurology*, 2005, 65, 1306–1308.
8. Chuang, Y. C. *et al.*, *J. Urol.*, 2006, **175**, 1158–1163.
9. Jankovic, J., *J. Neurol. Neurosurg. Psychiatry*, 2004, **75**, 951–957.
10. Aoki, K. R., *Neurotoxicology*, 2005, **26**, 785–793.
11. Simpson, L. L., *Ann. Int. Med.*, 1996, **125**, 616–617.
12. Narenda Pandya and Sonal Pandya – Sheth, *Botox, A Ready Reckoner*, VSSU Graphics, Mumbai, 2005, p. 23.
13. Jankovic, J. and Brin, M. F., *N. Engl. J. Med.*, 1991, **324**, 1186–1194.
14. Jankovic, J., *J. Neurol. Neurosurg. Psychiatry*, 2004, **75**, 951–957.
15. Piovesan, E. J., Teive, H. G., Kowacs, P. A., Della Coletta, M. V., Werneck, L. C. and Silberstein, S. D., *Neurology*, 2005, **65**, 1306–1308.
16. Chiang, Po-Hui and Hsien, Kaohsiung, *ScienceDaily*, 24 May 2007; http://www.sciencedaily.com
17. Chuang, Y. C., Huang, C. C., Kang, H. Y., Chiang, P. H., Demiguel, F. and Yoshimura, N., *J. Urol.*, 2006, **175**, 1158–1163.

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