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REVIEW

Botulinum neurotoxin A: A review

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Summary Despite its ubiquity in cosmetic circles and broad general awareness, a literature search of *botulinum neurotoxin* in JPRAS and BJPS yielded a mere 4 articles germane to cosmesis. A pair each detailing its application in masseteric hypertrophy[1,2] and the use of cryoanalgesia.[3,4] Given that botulinum neurotoxin A is the most commonly used cosmetic treatment, with American figures being most accurate,[5] a review of the background, development and scientific evidence would be perhaps useful, if not overdue, as Plastic Surgeons increasingly incorporate non-surgical interventions into their practices as part of a comprehensive facial rejuvenation strategy.

Introduction & historical background

The use of botulinum neurotoxin A (BoNT-A) for medical and cosmetic applications has enjoyed a short, but remarkable, life so far despite considerable inherent toxicity. Based on its estimated inhalational lethal dose, a single gram is reportedly sufficient to kill one million people.[6] Therapeutic BoNT-A was born in the 1970s originally as a non-surgical alternative for strabismus and the list of medical uses has mushroomed. Its cosmetic infancy arose from the serendipitous observation of diminished wrinkles during treatment for blepharospasm. Now, many millions of injections later, its safety and role have been clarified and it sits in, relatively, comfortable adulthood as an increasingly (Figure 1) routine part of the aesthetic regimen for many both in and out of the public eye. New uses continue to emerge, many of which have arisen in a similarly fortuitous fashion.

The storey of BoNT-A starts with its description by the German, Justinus Kerner, sometime between 1817 and 1822.[7] Perhaps combining his dual professions of poet and physician, he named it 'sausage poison', having observed that the toxin tended to thrive in poorly-prepared meat products, particularly those self-canned. Half a century later, his compatriot Müller Latinised sausage into "botulus" — to name the disease it produced ie., *botulism*. The causative bacterium, *Clostridium botulinum*, was first cultured by van Ermengem in 1897[8] and neuromuscular blockade as the mechanism of action was elucidated in 1949.[9] The potential role of BoNT-A as a bioterrorism agent

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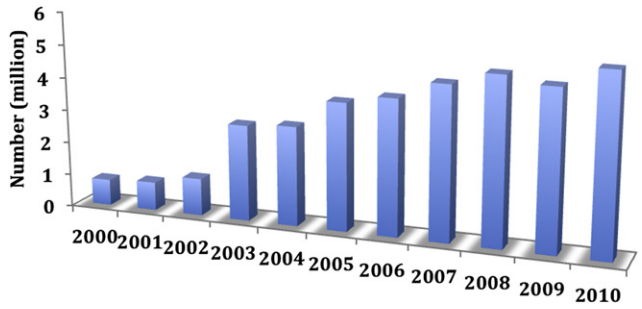

Figure 1 Figures for Botulinum neurotoxin A procedures in the US over the past 11 years (2000—2010) according to the American Society of Plastic Surgeons.[5]

has been discussed at governmental level[6] and it is rumoured that the Gestapo commander Reinhard Heydrich lost his life to a BoNT-A-containing grenade.[10] Fidel Castro was more adept at surviving the persistent attention of the CIA: Operation Mongoose reputedly included the impregnation of a box of his favourite Cohiba cigars with BoNT-A.[11] Southend earned a claim to fame with Bushara and Park's report of the first extra-somatic use of BoNT-A: autonomic chemodenervation for the amelioration of hyperhidrosis.[12]

Considered the father of medical BoNT-A, Alan Scott extended his primate studies and made the somewhat brave first injection into a human with strabismus.[13] He moved outside the orbit to treat patients with the highly disabling condition of blepharospasm in 1985[14] and Botox® Medical was accordingly FDA (Food and Drug Administration)-approved in 1989 for the treatment of strabismus, blepharo-and hemifacial-spasm. Table 1 lists some of the other regulatory milestones.

As to the first use of botulinum neurotoxin for cosmetic reasons, there is somewhat of a 'bun-fight'. Most authors cite the husband-and-wife Carruthers team's 1992 paper of their observation of an amelioration in local skin wrinkling whilst using BoNT-A for blepharospasm and they certainly reported the first scientific study.[15] Whilst Clark laid retrospective claim,[16] based on its use in symmetrising a contralateral frontal branch paralysis post-facelift in 1989,[17] in truth he did not really highlight the cosmetic association between muscle paralysis and skin wrinkles so it was left to the Carruthers to fully appreciate and take advantage of their own 'penicillin' moment. FDA approval, for Botox® Cosmetic, was granted in April 2002, but was, and remains, limited to the glabellar area. It is an important, but under-appreciated fact that all other areas are treated in an 'off-label' fashion.

Table 1 Some important dates in the regulatory evolution of BoNT-A.

Year	
1989 (December)	FDA approval for strabismus, blepharospasm and hemifacial spasm (Botox® Medical)
2000 (December)	FDA approval for cervical dystonia (Botox® Medical)
2002 (April)	FDA approval for moderate/severe glabellar line $\leq$ 65 years (Botox® Cosmetic)
2004 (July)	FDA approves Botox® for axillary hyperhidrosis
2006 (March)	MHRA licence for Vistabel® (Allergan) for moderate/severe glabellar lines $\leq$ 65 years when psychological impact exists
2009 (March)	Licence as above for Azzalure® (Dysport)
2009 (July)	FDA approves Dysport®
2009 (July)	FDA approval for Xeomin®
2010 (Oct)	FDA approves Botox® for migraine

Taxonomy and physiology

Clostridium botulinum is an anaerobic, Gram + ve, spore-forming bacillus, which produces a potent, neurologically-directed exotoxin. Eight serological types (A, B, C_1, C_2, D, E, F and G) are recognised, based on the antigenic specificity of each exotoxin (Table 2).[18] They share amino acid sequence, structural and functional commonalities and all act on the different parts of the same target receptor, bar C_2, which is not a neurotoxin. Interestingly, whilst very similar to tetanus, botulin toxins exert the diametrically opposite effect of a flaccid paralysis contrary to the muscle stiffness and spasm that prompted the lay term 'lockjaw'. Serotypes A and B are the only forms commercially available and, whilst they have very similar functions, they are antigenically dissimilar, which allows those very few who have developed antibodies to still benefit from neurotoxin treatment.

Biochemically, BoNT-A is a complex structure with the chemical formula $\mathbf{C_{6760}H_{10447}N_{1743}O_{2010}S_{32}}$ (Figure 2) and molecular weight of 150 kDa. The polypeptide comprises 2 chains: one light (50 kDa) and one heavy (100 kDa) conjoined by a disulphide bond, which is disrupted upon toxin activation. BoNT-A exists *in vivo* in complex form being surrounded by a coat of haemagglutinin proteins that protect it from being destroyed by the highly acidic environment of gastric juices when ingested. After absorption and a corresponding rise in pH these proteins release the neurotoxin to cause the well-known effects of botulism. Food-borne (as distinct from wound and infant) botulism is a severe food poisoning caused by ingesting foods containing the neurotoxins (usually A, B and E) formed during growth of the organism. Whilst the spores are heat-resistant and can survive in inadequately processed foods, the heat-labile toxin is denatured by boiling.[19] Although uncommon, botulism is of considerable concern because of its high mortality if not treated with urgency. Symptoms of foodborne botulism usually occur 18—36 h after ingestion and include marked lassitude, weakness and vertigo, usually followed by double vision and progressive difficulty in speaking and swallowing. Difficulty in breathing, weakness of other muscles, abdominal distension, and constipation are also common. It is important, however, to appreciate that BoNT-A has different consequences when injected into the skin and the standard vial of BoNT-A has 2×10^{-8}, or 200 million times, less than the lethal dose.[20] The most frequent vehicles for human botulism comprise

Table 2 Characteristics of the botulinum serotypes and their molecular targets.

Serotype	Potency	Duration	Features	Identified in	Molecular target
A	Highest	Longest 4–6 months	Dysport®, Botox®, Xeomin®	Human	SNAP-25
B	1/200th A	6 weeks	Neurobloc or Myobloc	Human	VAMP1,2 cellubrevin
Cα	1/10th A		Similar to A	Chicken	Syntaxins 1–3, SNAP-25
Cβ	1/10th A			Chicken	Syntaxins 1–3, SNAP-25
D				Cattle	VAMP1,2 cellubrevin
E				Human	SNAP-25
F		4–5 weeks		Human	VAMP1,2 cellubrevin
G				Soil	VAMP1,2 cellubrevin

sausages, meat products, canned vegetables and seafoods as the organism is widely distributed in soils, sediments of streams, lakes, and coastal waters the intestinal tracts of fish and mammals, and the gills and viscera of shellfish.

The philosophy underlying treatment with BoNT-A is relatively straightforward. One targets the hyperkinetic muscles beneath the wrinkles so a good functional knowledge of facial muscle anatomy is required. Following injection it diffuses

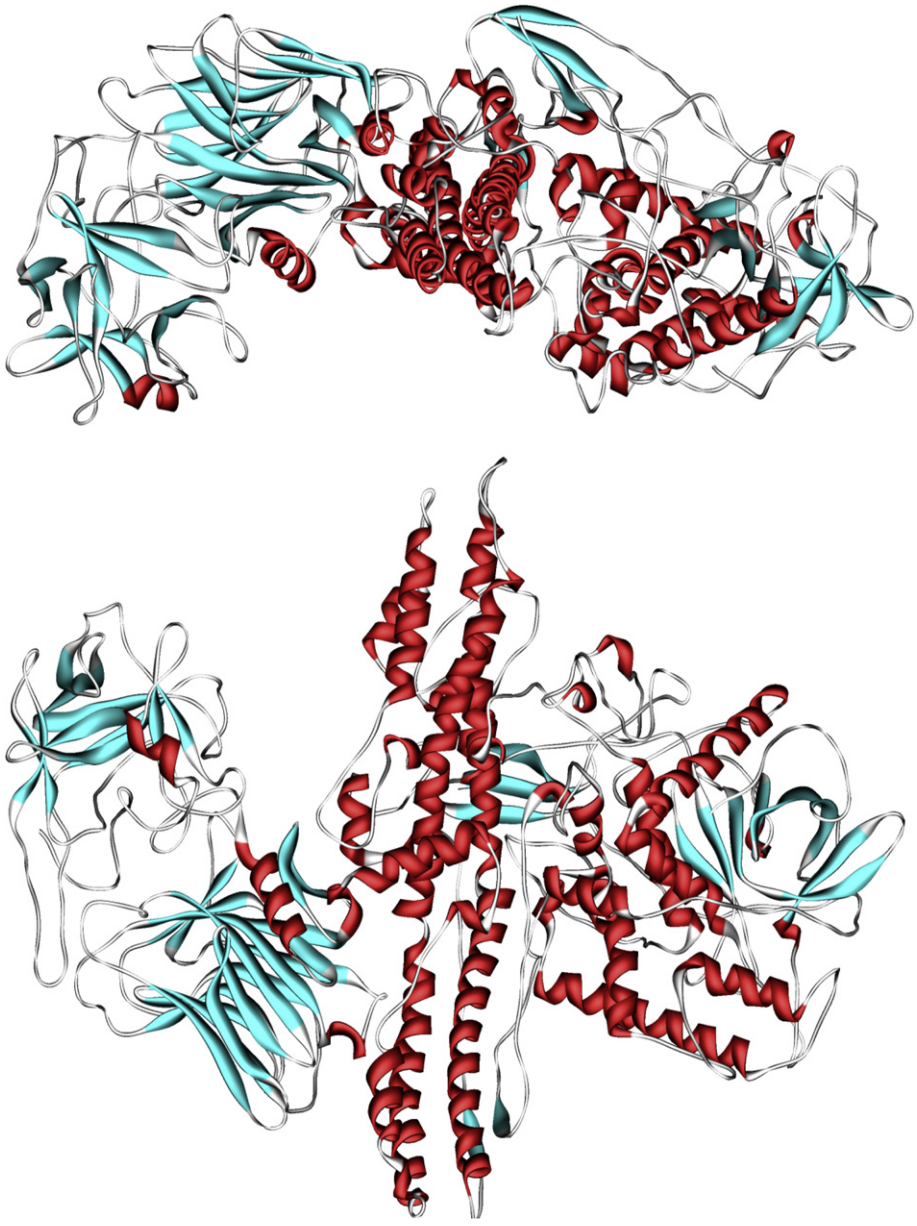

Figure 2 The structure of botulinum neurotoxin A.

and, through attachment of the heavy chain, binds to the axon terminal surface: this process taking approximately 30 min. The heavy chain then forms a channel through which the light chain passes as the disulphide bond weakens.[21] With its zinc-dependent proteolytic activity, the 50 kDa moiety specifically degrades cytosolic SNAP (soluble N-ethyl-maleimide-sensitive fusion-attachment protein)-25. SNAP receptors are found on both vesicle and plasma membranes and are essential for acetylcholine release from the pre-synaptic terminal of the neuromuscular junction (NMJ).[22] All of this takes time and explains why it takes 2–3 days to appear. The paralytic effect of BoNT-A is dose-related with the peak effect occurring between 5 and 8 days.[23] This chemical denervation is permanent and histopathological examination of treated muscle shows atrophy with mild demyelination at the neuron terminal.[18] The reason for the loss of effect after 3–6 months has been shown to be due to synaptic switching[24] and neurogenic axonal sprouting.[25] Although perceived irksome due to the need for regular and repeat administration, this is one of the most important facets of BoNT-A treatment - its reversibility. It is well to remember that any adverse effects will wear off too in time.

The duration of effect is somewhat variable, lasting 3–5 months, but the benefits have been observed to increase with time and lead to a diminished production of wrinkles.[26] It is also believed that a degree of conditioning occurs, that is, regular injections elongate the duration of effect leading to correspondingly longer intervals between treatments. Whether this is consequential upon behavioural change, retarded neural regeneration or true muscular atrophy is unknown.[20,27] Anecdotally, this may occur if the effect has not entirely worn off before re-treatment, but formal study is required. With BoNT-A acting at the muscular level, the individual's muscle mass will have a direct bearing on response. It is therefore no surprise that males, with a generally greater muscle mass, require more neurotoxin for a similar effect.[28] Less intuitively, thicker skin, for example found in Asians who have more collagen, require more BoNT-A for an equivalent effect to their thinner-skinned Caucasian counterparts.[29]

Safety and complications

The adverse events of BoNT-A are essentially predictable and stem from either an unwanted or excess effect of the neurotoxin itself or an allergic reaction to the protein structure, hence the relative contraindication in those with an egg allergy. According to one of the original Botox gurus, Alastair Carruthers, there are really only two significant complications of correct neurotoxin administration: headaches and eyelid ptosis.[30] The former tend to abate with regular administration and the latter reduce in frequency with injector experience. In a meta-analysis performed in 2004, mild-to-moderate complications occurred in 25%. Whilst this may sound high, placebo treatment caused 15% and both involved focal weaknesses that entirely reversed in time.[31]

Adverse effects: systemic

The scale of potency (toxicity) employed for evaluating the safety of drugs and therapeutic products is the lethal dose 50 (LD_{50}), which refers to the quantity that causes death in 50% of test subjects. For obvious reasons this has never been performed in humans, and a murine assay is used for BoNT-A. One mouse unit (mU) kills 50% of a group of 18–22 g Swiss Webster mice following intraperitoneal injection. For a 70 kg adult the LD_{50} is estimated, from primate studies, to be 40 u/kg or 2800U.[32] Given that 100U and 300-600U are used for cosmetic and medical indications respectively, the safety margins are therefore wide.

The FDA reviewed all serious adverse events (AE) between 1989 and 2003 and reported a death toll of 28, 4 of them children (Table 3). None of the deaths, however, occurred in cosmetic uses and 26 were adjudged to have some underlying systemic disease carrying an elevated risk of mortality. 'Serious' AEs occurred 33-times more frequently in therapeutic uses where the median doses were 100 and 25BU respectively.[33] A further fatality has resulted from off-label BoNT-A use for neck pain: in this case a female died after what was considered to be an anaphylactic reaction. It was adjudged that the causative agent was the BoNT-A-lignocaine mixture rather the BoNT-A itself.[34] There have also been reports of unlicensed, 'black-market' preparations and, obviously, unscrupulous practitioners. One particular case in 2004 perhaps demonstrated *karma* when a suspended Floridian doctor, by the name of Bach McComb, who not only injected a couple of patients, but also his girlfriend and himself. All required artificial ventilation for systemic botulism and were lucky to survive.[35] It transpired that he used a non-approved and illegal formulation that may have contained as much as 10 million Botox Units (BU) — the standard Allegan vial contains only 100 BU.

Whilst the media are quick to highlight any suggestion of neurotoxin dissemination beyond the target muscles, to date such evidence has been sparse, well-documented and of negligible clinical relevance.[23,36] A more recent study, in rabbits injected with medical-, rather than cosmetic-level, doses demonstrated atrophy and loss of contractile tissue in non-injected limbs, apparently due to BoNT-A crossing the membrane barrier, but the significance remains unclear.[37]

Adverse effects: lack of response

Some patients with medical indications including cervical dystonia have become immune to further injections — 'secondary non-responders' — and this has been blamed on an antibody-mediated immune response.[38] Various figures have been presented, but a recent meta-analysis yielded a rate of 0.49% across 2240 subjects.[39] Interestingly, only 3 of the 11

Table 3 Causes of death in adverse events reported to the US FDA.[33]

Cause	Number
Respiratory arrest	6
Myocardial infarction	5
Cerebrovascular accident	3
Pulmonary embolism	2
Pneumonia	2
'Other' known	5
'Other' unknown	5

with documented antibodies were actually clinically unresponsive. Another study of 503 secondary non-responders was able to confirm neutralising antibody presence in only 44.5% so the aetiology of loss of efficacy is not antibody-mediated in over half.[40] This study also correlated non-responsiveness with both greater dose and higher frequency. It is extremely uncommon in cosmetic practice as the doses are much lower, the sole reported instance to date being the identification of antibodies in a patient who had received a total of 240 botox Units for masseteric hypertrophy.[41] For treatment, the usual practice is trial of a different serotype, usually B, proven in both dystonia[42] and glabellar lines.[43] BoNT-B has demonstrated efficacy and comparable safety to BoNT-A in the glabellar region, however, its duration is much shorter at 8 weeks and the mild acidity renders injections less comfortable.[44] On the other hand its speed of onset, 2–3 days, is double that of BoNT-A.

Adverse effects: localised

Because adverse effects include bruising it is important to use the smallest needle possible (31 gauge) and avoid any superficial vessels, so good lighting and anatomical knowledge are important. Some find the injection unpleasant, but the use of topical anaesthesia has not been equivocally proven helpful. Topical EMLA produced a measurable and statistically significant amelioration of injection pain in two studies,[45,46] but the combination of betacaine with cryoanalgesia had no beneficial impact and adversely affected the neurotoxin effect in another.[47]

The headache that often occurs for the initial 24 h was previously believed due to the neurotoxin itself, but meta-analysis of placebo studies indicates statistical indistinguishability so it appears to relate to the injection itself.[48] Headaches also become far less common with repeat injections so patients can be reassured.[30] A cluster of 5 cases of severe, intractable headache reported in 2002[49] seems not to have been repeated elsewhere and their significance remains unclear.

Blepharoptosis is often cited, but simply refers to a spread of the effect beyond that intended. If neurotoxin diffuses when injecting the corrugator supercilii, the upper eyelid may droop. This can be avoided by careful technique, digital pressure on the orbital rim to limit toxin diffusion and avoiding post-injection massage. The formulation of BoNT-A may also be important as there is evidence that Dysport® diffuses more than Botox®.[50] An area that has received attention is limiting the amount of neurotoxin through what is known as the ultra-concentrated technique.[51] Instead of diluting in 2.5 mL, a single mL is used. Reported benefits include greater comfort and less post-injection distortion.[52] Finally, it has been suggested that the higher the volume, the greater the diffusion[53] and the shorter the duration of effect.[54] In any case, the effect tends to be short-lived and is often ameliorated by topical α_2-adrenoceptor agonists such as 0.5% apraclonidine.[55]

Botulinum toxin clinical use

As is frequently the case, the first to market with a novel product often becomes the descriptive noun and Allergan's BoNT-A, Botox® (Allergan, Marlow International, Bucks., UK: data insert[56]), has effectively eponymised the toxin just as Hoover did with their vacuum cleaner. Whilst America is predominantly Botox territory, Europeans have had Dysport (Ipsen Biopharm Ltd., Wrexham, UK: data insert[57]) for almost as long, but there has been a recent entrant to the market – Xeomin, a 'naked' BoNT-A (Merz Pharmaceuticals GmbH, Frankfurt, Germany: data insert[58]). Whilst previously it had been thought that the complexing proteins played an important role, it is now known that dissociation is virtually instantaneous at physiological pH so a reduced protein load is a theoretical advantage. Whilst the manufacturers are keen to maintain secrecy over their precise formulations and, indeed, emphasise differences where they pertain to improved efficacy, predictability or safety for proprietary reasons, it does make it difficult for practitioners to make logical comparisons and assess things on a scientific basis. In essence, all share the 150 kDa neurotoxin, but different associated proteins. Table 4 provides a summary of the pertinent characteristics. All three companies provide their BoNT-A in two volumes, the smaller being intended for single patient use.

There has been much research directed towards the comparative potencies of Botox® and Dysport®. Overall, the literature suggests a conversion factor of 2.5–4 × Units of Dysport® compared to Botox®,[59–61] however, because BU and DU are proprietary measures, any conversion is an estimate. Botox® Cosmetic and Xeomin® are interchangeable due to the instantaneous dissociation of the former, which behaves identically to the naked BoNT-A.[62] Complexing proteins are an important ingredient because whilst Dysport® disperses significantly more than the other two,[50] it also lasts significantly longer, at a 2.5:1 ratio.[61]

In terms of scientific studies, Botox has been investigated predominantly in the US and Dysport in the UK and Europe, particularly by Ascher.[28] Direct comparison of exact equivalence between the two is often precluded by different outcome measures, for example, maximal frown in preferred in the US whilst the resting state is favoured by Europeans[63] in addition to different units by manufacturer. Finally, with current assessment being subjective and clinically-based, a new objective method, using silicone masks and standardised photography[64], may be particularly helpful with the crowded market or dose comparisons and trials between different preparations.

Both Allegan and Ipsen recommend storage of the raw and reconstituted vials at below 4 °C. Merz recommend storage below 4 °C after reconstitution, but only below 25 °C before and this allows more convenient transport. Although reconstituted toxin should be disposed of after 4 h[56,57] as efficiency is said to reduce after this time, several studies have shown maintained efficacy up to 4–6 weeks.[65,66] Reconstitution, where required, was thought to require care to protect the fragile toxin, but there has been shown to be no reduced efficiency with vigorous agitation,[67] although this produces more air bubbles so increases drawing up time. Despite data sheets recommending non-preserved saline, there is some evidence that using preserved isotonic saline as the diluent is less painful on injection.[68]

Table 4 Summary table of some individual characteristics of the currently available botulinum neurotoxins.

Manufacturer	Chemical	Proprietary	Molecular weight (kDa)	pH	Carrier	Units/vial	Preparation	Product	Storage	Comments
Allergan	OnabotulinumtoxinA	Botox® Cosmetic Vistabel	900	7.2	0.5 mg human albumin 0.9 mg sodium chloride	100 u 50 u	Acid precipitation	Crystalline powder Vacuum-dried	4 h	Virtually immediate dissociation
Ipsen	AbobotulinumtoxinA	Dysport®	750	7.2	0.125 mg human albumin 2.5 mg lactose	500 u 125 u	Column-based purification	Crystalline powder Lyophilised	4 h	Wider dispersion; longer duration
Merz	IncobotulinumtoxinA	Xeomin	150		1 mg human albumin	100 u	Column-based purification	Sucrose, albumin (h)	Room temperature 36 months	No complexing proteins
		Bocouture			4.7 mg sucrose	50 u				No need to refridgerate so good for travel
Solstice	RimabotulinumtoxinA	NeuroBloc (UK) MyoBloc (US)	500–700	5.6	0.05% human albumin 0.1 M sodium chloride	5000/ml		Pre-mixed	2 yrs (at 4–8 °C)	Much shorter duration than BoNT-A

Non-cosmetic uses

The therapeutic use of neurotoxin is hardly the new, and untried, phenomenon sometimes portrayed by the media and the medical indications are legion[69] with the predominant mechanism of action being cholinergic neurotransmitter interruption. Moreover, medical uses generally involve significantly higher doses than cosmetic and even by 1989 Scott was able to review the potential complications and conclude them to be 'rare, mild and treatable' in the main.[70] Novel avenues include chronic pain — through non-cholinergic neurotransmitters, including substance P, CGRP and possibly a neurotoxin degradation product.[71] An intriguing role for BoNT-A in capsular contracture around mammary prostheses has been suggested by a study of its injection into pectoralis major at the time of implantation.[72] Whilst the precise mechanism is unclear, BoNT-A is known to inhibit fibroblast proliferation. It may, of course, hint at a role for the muscle itself in adverse capsular contracture generation.

Although beyond the scope of this review, there are some novel avenues of BoNT-A research that are worth mention. Serendipity is a common theme in the evolving BoNT-A story from Jean Carruthers' first observation[15] to its recent (October 2010) FDA approval for the treatment of migraine-type headaches. Credit on this occasion is taken by the Plastic Surgeon, Guyuron, who observed a link between BoNT-A administration and migraine alleviation in the mid-1990s.[73] The precise mechanism of action in this condition, however, has yet to be completely elucidated other than 'trigger points'. Although these fulfil one of Koch's Postulates ie., that of their removal alleviating the condition, unequivocal confirmation is awaited. This is exemplified in a recent, randomised, double-blind study where, although patients' assessment demonstrated a clear difference between BoNT-A and placebo, there was only a trend observed for absolute attack count.[74]

The latest and most intriguing avenue of research involves the psychological indications for BoNT-A. Recent studies suggest an anti-depressant role whereby emotions are reverse-engineered. This is based upon the hypothesis that the brain is constantly aware of what the body is doing, through continuous feedback, in the same way as someone forced to smile even when unhappy will feel better - the "facial feedback hypothesis".[75] An experimental study suggests that cosmetic use of botulinum toxin for treatment of glabellar lines affects human cognition. Havas et al[76] asked participants to read emotional (angry, sad and happy) sentences before and two weeks after BoNT-A injections in the corrugator supercilii. Reading times for angry and sad sentences were longer after injection, while reading times for happy sentences were unchanged. In a double-blind, randomised study BoNT-A was shown to not only improve quality of life and self-esteem, but that such gains were sustained even if later injected with placebo.[77]

One might wonder at the link between neurotoxin-induced facial muscle paralysis for wrinkle reduction and baldness, but a Californian surgeon has claimed one. Apparently noted initially as a consequence of injecting his mother's scalp for the treatment of chemotherapy-induced headaches, studies on other volunteers have convinced one

physician.[78] He combines the injections with a vitamin cocktail and suggests that it works through reducing tension on the scalp muscles. This improved circulation allows more nutrients to stimulate hair cell growth. Whilst encouraging, this technique requires further, independent testing to confirm both safety and efficacy.

Finally, new transdermal preparations, RT001[79] and RT002[80] are currently being assessed for delivery of BoNT-A without the need for injections. Whilst on first sight an apparent Holy Grail, it may well work only with thin-skinned areas, has the potential for unequal treatment and could potentially produce even fewer properly trained people administering BoNT-A.

Conclusion

And so, with the benefit of millions of injections, a very limited adverse effect profile and lower mortality than many surgical procedures, botulinum neurotoxin therapy has become an entrenched part of rejuvenation. Our American counterparts have more experience with it cosmetically, and are able to provide comprehensive reviews of patient-reported satisfaction showing not only that it is high, but consistently so.[81] Despite the burgeoning uses touched upon above, botulinum toxin is not a panacea, but does seem to have a remarkably wide range of beneficial effects. Although latecomers to the BoNT-A party, aesthetic surgeons draw on a wide and comfortable familiarity with the muscles of facial expression so should be at the vanguard of BoNT-A therapy.

Conflict of interest

Neither author has any conflict of interest to declare.

References

1. To EW, Ahuja AT, Ho WS, et al. A prospective study of the effect of botulinum toxin A on masseteric muscle hypertrophy with ultrasonographic and electromyographic measurement. *Br J Plast Surg* 2001;**54**:197—200.
2. Gaofeng L, Jun T, Bo P, et al. Evaluation and selecting indications for the treatment of improving facial morphology by masseteric injection of botulinum toxin type A. *J Plast Reconstr Aesthet Surg* 2010;**63**:2026—31.
3. Cerquiero J, Matti B. Beneficial use of an ice-balloon as part of botulinum toxin type A therapy for facial dynamic wrinkles and other skin injection procedures. *Br J Plast Surg* 2003;**56**:619.
4. Engel SJ, Afifi AM, Zins JE. Botulinum toxin injection pain relief using a topical anesthetic skin refrigerant. *J Plast Reconstr Aesthet Surg* 2010;**63**:1443—6.
5. http://www.plasticsurgery.org/Documents/news-resources/statistics/2010-statisticss/Overall-Trends/2010-cosmetic-plastic-surgery-minimally-invasive-statistics.pdf; [accessed 06.11.11].
6. Arnon SS, Schechter R, Inglesby TV, et al. Botulinum toxin as a biological weapon: medical and public health management. *JAMA* 2001;**285**:1059—70.
7. Erbguth FJ. Historical notes on botulism, Clostridium botulinum, botulinum toxin and the idea of the therapeutic use of the toxin. *Mov Disord* 2004;**19**:S2—6.
8. van Ermengen EP. Über einen neuen anaëroben Bacillus and seine Beziehungen zum Botulismus. *Z Hyg Infektionskrankh* 1897;**26**:1—56.
9. Burgen ASV, Dickens F, Zatman LJ. The action of botulinum toxin on the neuro-muscular junction. *J Physiol* 1949;**109**:10—24.
10. Harris R, Paxman J. *A higher form of killing: the secret history of chemical and biological warfare*. London: Chatto & Windus; 1982.
11. http://news.bbc.co.uk/1/hi/world/americas/244974.stm; [accessed 06.11.11].
12. Bushara KO, Park DM. Botulinum toxin and sweating. *J Neurol Neurosurg Psychiatry* 1994;**57**:1437—8.
13. Scott AB. Botulinum toxin injection into extraocular muscles as an alternative to strabismus surgery. *Ophthalmology* 1980;**87**:1044—9.
14. Scott AB, Kennedy RA, Stubbs HA. Botulinum toxin A injection as a treatment for blepharospasm. *Arch Opthalmol* 1985;**103**:347—50.
15. Carruthers JD, Carruthers JA. Treatment of glabellar frown lines with C. botulinum-A exotoxin. *J Dermatol Surg Oncol* 1992;**18**:17—21.
16. Clark RP, Berris CE. Botulinum toxin: a treatment for facial asymmetry caused by facial nerve paralysis: follow up. *Plast Reconst Surg* 2005;**115**:573—4.
17. Clark RP, Berris CE. Botulinum toxin: a treatment for facial asymmetry caused by facial nerve paralysis. *Plast Reconst Surg* 1989;**84**:353—5.
18. Osako M, Keltner JL. Botulinum A toxin (Oculinum) in ophthalmology. *Surv Ophthalmol* 1991;**36**:28—46.
19. Setlow P. I will survive: DNA protection in bacterial spores. *Trends Microbiol* 2007;**15**:172—80.
20. Cartee TV, Monheit GD. An overview of botulinum toxins: past, present, and future. *Clin Plast Surg* 2011;**38**:409—26.
21. Koriazova LK, Montal M. Translocation of botulinum neurotoxin light chain protease through the heavy chain channel. *Nat Struct Biol* 2003;**10**:13—8.
22. Schiavo G, Matteoli M, Montecucco C. Neurotoxins affecting neuroexocytosis. *Physiol Rev* 2000;**80**:717—66.
23. Sanders DB, Massey EW, Buckley EG. Botulinum toxin for blepharospasm: single-fibre EMG studies. *Neurology* 1986;**36**:545—7.
24. de Paiva A, Meunier FA, Molgo J, Aoki KR, Dolly JO. Functional repair of motor endplates after botulinum neurotoxin type A poisoning: biphasic switch of synaptic activity between nerve sprouts and their parent terminals. *Proc Natl Acad Sci USA* 1999;**96**:3200—5.
25. Angaut-Petit D, Molgó J, Comella JX, Faille L, Tabti N. Terminal sprouting in mouse neuromuscular junctions poisoned with botulinum type A toxin: morphological and electrophysiological features. *Neuroscience* 1990;**37**:799—808.
26. [Chapter 5] pp. 83—92 In: Burgess CM, editor. *Cosmetic dermatology*. Heidelberg: Springer; 2005.
27. Fagien S. Botox for the treatment of dynamic and hyperkinetic facial lines and furrows: adjunctive use in facial aesthetic surgery. *Plast Reconstr Surg* 2003;**112**(Suppl.):40S—52S.
28. Ascher B, Zakine B, Kestemont P, Baspeyras M, Bougara A, Santini J. A multicentre, randomized, double-blind, placebo-controlled study of efficacy and safety of 3 doses of botulinum toxin A in the treatment of glabellar lines. *J Am Acad Dermatol* 2004;**51**:223—33.
29. Ahn KY, Park MY, Park DH, Han DG. Botulinum toxin A for the treatment of facial hyperkinetic wrinkle lines in Koreans. *Plast Reconstr Surg* 2000;**105**:778—84.
30. Carruthers A. Problems with toxins. *Body Lang* 2010;**35**:43—4.
31. Naumann M, Jankovic J. Saftey of botulinum toxin type A: a systematic review and meta-analysis. *Curr Med Res Opin* 2004;**20**:981—90.

32. Scott AB, Suzuki D. Systemic toxicity of botulinum toxin by intramuscular injection in the monkey. *Mov Disord* 1988;**3**: 333—5.
33. Coté TR, Mohan AK, Polder JA, Walton MK, Braun MM. Botulinum toxin type A injections: adverse events reported to the US Food and Drug Administration in therapeutic and cosmetic cases. *J Am Acad Dermatol* 2005;**53**:407—15.
34. Li M, Goldberger BA, Hopkins C. Fatal case of BOTOX-related anaphylaxis. *J Forensic Sci* 2005;**50**:169—72.
35. http://www.telegraph.co.uk/news/worldnews/northamerica/usa/1478282/Doctor-and-patients-fight-for-life-after-anti-wrinkle-jabs.html; [accessed 06.11.11].
36. Claus D, Druschky A, Erbguth F. Botulinum toxin: influence on respiratory heart rate variation. *Mov Dosord* 1995;**10**:574—9.
37. Fortuna R, Vaz MA, Youssef AR, Longino D, Herzog W. Changes in contractile properties of muscles receiving repeat injections of botulinum toxin (Botox). *J Biomech* 2011;**44**:39—44.
38. Kessler KR, Skutta M, Benecke R. Long-term treatment of cervical dystonia with botulinum toxin A: efficacy, safety and antibody frequency. *J Neurol* 1999;**246**:265—74.
39. Naumann M, Carruthers A, Carruthers J, et al. Meta-analysis of neutralizing antibody conversion with onabotulinumtoxinA (BOTOX®) across multiple indications. *Mov Disord* 2010;**15**(25): 2211—8.
40. Lange O, Bigalke H, Dengler R, Wegner F, deGroot M, Wohlfarth K. Neutralizing antibodies and secondary therapy failure after treatment with botulinum toxin type A: much ado about nothing? *Clin Neuropharmacol* 2009;**32**:213—8.
41. Lee SK. Antibody-induced failure of botulinum toxin type A therapy in a patient with masseteric hypertrophy. *Dermatol Surg* 2007;**33**:S105—10.
42. Brin MF, Lew MF, Adler CH, et al. Safety and efficacy of NeuroBloc (botulinum toxin type B) in type A-resistant cervical dystonia. *Neurology* 1999;**53**:1431—8.
43. Alster TSL, Lupton JR. Botulinum toxin type B for dynamic glabellar rhytides refractory to botulinum toxin type A. *Dermatol Surg* 2003;**29**:516—8.
44. Flynn TC, Clark RE. Botulinum toxin type B (MYOBLOC) versus botulinum toxin type A (BOTOX) frontalis study: rate of onset and radius of diffusion. 2nd. *Dermatol Surg* 2003; **29**:519—22.
45. Söylev MF, Koçak N, Kuvaki B, Ozkan SB, Kir E. Anaesthesia with EMLA cream for botulinum A toxin injection into eyelids. *Ophthalmologica* 2002;**216**:355—8.
46. Eppley BL. Easing Botox administration with EMLA cream. *Aesthet Surg J* 2004;**24**:79—81.
47. Sami MS, Soparka CNS, Patrinely JR, Hollier LM, Hollier LH. Efficacy of Botulinum toxin type A after topical anaesthesia. *Ophthal Plast Reconstr Surg* 2006;**22**:448—545.
48. Brin MF, Tl Boodhoo, Pogoda JM, et al. Safety and tolerability of onabotulinumtoxinA in the treatment of facial lines: a meta-analysis of individual patient data from global clinical registration studies in 1678 participants. *J Am Acad Dermatol* 2009; **61**:961—70.
49. Alam M, Arndt KA, Dover JS. Severe, intractable headache after injection with botulinum A exotoxin: a report of 5 cases. *J Am Acad Dermatol* 2002;**46**:62—5.
50. Kerscher M, Roll S, Becker A, Wigger-Alberti W. Comparison of the spread of three botulinum toxin type A preparations. *Arch Dermatol Res* 2011 Oct {Epub ahead of print}.
51. Klein AW. Dilution and storage of botulinum toxin. *Dermatol Surg* 1998;**24**:1179—80.
52. Carruthers A, Carruthers J, Cohen J. Dilution volume of botulinum toxin type A for the treatment of glabellar rhytides: does it matter? *Dermatol Surg* 2007;**33**:S97—104.
53. Hsu TS, Dover JS, Arndt KA. Effect of volume and concentration on the diffusion of botulinum exotoxin A. *Arch Dermatol* 2004; **140**:1351—4.
54. Klein AW. Complications and adverse reactions with the use of botulinum toxin. *Dis Mon* 2002;**48**:336—56.
55. Scheinfeld N. The use of apraclonidine eyedrops to treat ptosis after the administration of botulinum toxin to the upper face. *Dermatol Online J* 2005;**11**:9—11.
56. Allergan, Inc. Irvine, CA, USA Botox® Cosmetic; package insert.
57. Ipsen Biopharm Ltd., Wrexham, UK; package insert.
58. Merz Pharmaceuticals GmbH, Frankfurt, Germany; package insert.
59. Lowe NJ. Botulinum toxin type A for facial rejuvenation. United States and United Kingdom perspectives. *Dermatol Surg* 1998;**24**:1216—8.
60. Klein AW, Carruthers A, Fagien S, Lowe NJ. Comparisons among Botulinum toxins: an evidence-based review. *Plast Reconstr Surg* 2008;**121**. 413e-22e.
61. Nestor MS, Ablon GR. Duration of action of AbobotulinumtoxinA and OnabotulinumtoxinA. *J Clin Aesthet Dermatol* 2011;**4**: 43—9.
62. Sattler G, Callander MJ, Grablowitz D, et al. Noninferiority of incobotulinumtoxinA, free form complexing proteins, compared with another botulinum toxin type A in the treatment of glabellar frown lines. *Dermatol Surg* 2010;**36**: 2146—54.
63. Monheit GD. Botulinum toxins: the new expanded market. *Plast Surg Pulse* 2009;**1**:1—6.
64. Branford OA, Dann SC, Grobbelaar AO. The quantitative assessment of wrinkle depth: turning the microscope on botulinum toxin type A. *Ann Plast Surg* 2010;**65**:285—93.
65. Garcia A, Fulton Jr JE. Cosmetic denervation of the muscles of facial expression with botulinum toxin. A dose-response study. *Dermatol Surg* 1996;**22**:39—43.
66. Hexsel DM, De Almeida AT, Rutowitsch M, et al. Multicentre, double-blind study of the efficacy of injections with botulinum toxin type A reconstituted up to six consecutive weeks before application. *Dermatol Surg* 2003;**29**(5):23—9.
67. Trindade de Almeida AR, Kadunc BV, Di Chiacchio N, Neto DR. Foam during reconstitution does not affect the potency of botulinum toxin type A. *Dermatol Surg* 2003;**29**:530—1.
68. Alam M, Dover JS, Arndt KA. Pain associated with injection of botulinum A exotoxin reconstituted using isotonic sodium chloride with and without preservative: a double-blind, randomised controlled trial. *Arch Dermatol* 2002;**139**:510—4.
69. Hackett R, Kam PCA. Botulinum toxin: pharmacology and clinical developments: a literature review. *Med Chem* 2007;**3**: 333—45.
70. Scott AB, Magoon EH, McNeer KW, Stager DR. Botulinum treatment of strabismus in children. *Trans Am Ophthalmol Soc* 1989;**87**:174—80.
71. Guyer BM. Mechanism of botulinum toxin in the relief of chronic pain. *Curr Rev Pain* 1999;**3**:427—31.
72. Xiao Z. Effect of botulinum toxin type A on the capsule around a subpectoral implant for breast augmentation. *Aesthet Plast Surg* 2009;**33**:782—3.
73. Guyuron B, Tucker T, Kriegler J. Botulinum toxin A and migraine surgery. *Plast Reconstr Surg* 2003;**112**. 171S—3S.
74. Petri S, Tolle T, Straube A, Pfaffenrath V, Stefenelli U, Ceballos-Baumann A. Botulinum toxin as preventive treatment for migraine: a randomised double-blind study. *Eur Neurol* 2009; **62**:204—11.
75. Finzi E, Wasserman E. Treatment of depression with botulinum toxin A: a case series. *Dermatol Surg* 2006;**32**:645—9.
76. Havas D, Glenberg A, Gutowski K, Lucarelli M, Davidson R. Cosmetic use of botulinum toxin-A affects processing of emotional language. *Psychol Sci* 2010;**21**:895—900.
77. Dayan SH, Arkins JP, Patel AB, Gal TJ. A double-blind, randomised, placebo-controlled health-outcomes survey of the effect of botulinum toxin type A injections on quality of life and self-esteem. *Dermatol Surg* 2010;**36**:2088—97.

78. http://www.telegraph.co.uk/health/healthnews/5609011/Botox-could-be-the-cure-for-baldness.html; [accessed 06.11.11].
79. Brandt F, O'Connell C, Cazzaniga A, Waugh JM. Efficacy and safety evaluation of a novel botulinum toxin topical gel for the treatment of moderate to severe lateral canthal lines. *Dermatol Surg* 2010;**36**:2111—8.
80. Stone HF, Zhu Z, Thach TQ, Ruegg CL. Characterisation of diffusion and duration of action of a new botulinum toxin type A formulation. *Toxicon* 2011;**58**:159—67.
81. Fagien S, Carruthers JDA. A comprehensive review of patient-reported satisfaction with botulinum toxin type A for aesthetic procedures. *Plast Reconstr Surg* 2008;**122**:1915—25.