Botulinum Toxin

Edited by
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Summary and Key Features

- Injectable BoNT-A has been successfully used across a range of medical disorders including strabismus, blepharospasm, focal dystonias, migraine, spasticity associated with juvenile cerebral palsy and adult stroke as well as various cosmetic treatments such as to temporarily relax hyperfunctional rhytides.
- Alternative methods have been developed due to limitations and disadvantages of injections of BoNTA which includes iontophoresis device-based approaches and topical liposomes and CPP drug delivery systems.
- Topical botulinum toxin delivery through the skin may offer opportunities to treat anatomical areas that are either difficult to manage with injectables or where patients may prefer to avoid injectables, and there may also be useful roles for topical botulinum therapies as adjunctive or extender therapies for the injectable techniques now in use.

Background

Injectable botulinum toxin type A (BoNT-A) has been successfully used across a range of medical disorders including strabismus, blepharospasm, focal dystonias, migraine, spasticity associated with juvenile cerebral palsy and adult stroke, and various cosmetic treatments. Thus, the safety and effectiveness of BoNT-A for treating these conditions have been well established over the past 20 years.

While aesthetic medicine has been inundated with treatments for facial rhytides, BoNT-A is the only Food and Drug Administration (FDA)-approved treatment to temporarily relax hyperfunctional rhytides. Since 1992, botulinum toxin has been used to treat a variety of cosmetic conditions and medical indications. Following the first description of the treatment for glabellar rhytides (‘frown line’ wrinkles) by Carruthers & Carruthers in 1992, botulinum toxin has revolutionized the practice of aesthetic medicine. In 2002, the United States (US) FDA approved the use of Botox® Cosmetic (botulinum toxin type A purified toxin complex; Allergan, Inc.) for the treatment of moderate-to-severe glabellar rhytides, associated with corrugator and/or procerus muscle activity in adult patients 65 years of age and younger. In 2004, Carruthers et al reported the consensus recommendations that BoNT-A is effective and safe, and patient satisfaction is high. By 2005, Botox® cosmetic injections were the most common non-invasive physician-administered cosmetic procedure worldwide.

Need for transcutaneous delivery systems

Matarasso & Matarasso reported in 2001 that the paralytic effect of injected BoNT-A can span up to 3 cm from the site of injection and even further when dilute concentrations and large volumes of BoNT-A are used. Limitations of injections of BoNT-A include pain, erythema, swelling, potential infection from needle use, and potential for reduced normal expression in the treated area. The patient’s pre-treatment medical regimen is potentially impacted by being advised to avoid aspirin, non-steroidal anti-inflammatory drugs, and vitamin E prior to injection, to reduce the risk of bleeding and bruising. Bruising is of particular concern in the crow’s feet (also known as lateral canthal lines or LCL), where the blood vessels are superficial and the skin is thin. Because of the disadvantage of requiring injection as the route of administration, alternative methods of drug delivery have been developed that can address some of these concerns.

Conventional transepidermal drug delivery systems can deliver only small molecules. Some examples of smaller molecules that can be delivered through the skin topically include progesterone, scopolamine, certain antibiotics, and nicotine. These are readily delivered in traditional topical preparations, creams, ointments, adhesive patches, etc. These systems range from very inefficient to completely ineffective in delivering biologically active proteins and other macromolecules across the skin barrier. The stratum corneum and upper layers of the epidermis are lipid-rich barriers to entry for most of the larger molecules, which is, after all, the skin’s primary function – to exclude assaults on the inner host by biologically active macromolecules. The flux of most proteins across the skin barrier is essentially zero.
Potential approaches to transcutaneous delivery of BoNT-A

Attempts to increase penetration by manipulation of drug structure have been largely ineffective for most proteins. For example, conjugation of a drug to a carrier can compromise drug activity and permeation enhancers may disrupt biological activity of the protein. At present, delivery of most macromolecules across the skin barrier requires direct injection (e.g., insulin, antibodies, toxins, and growth hormone).

Device-based approaches such as iontophoresis have been explored as well. Iontophoresis utilizes a different mechanism for drug delivery into the skin, but lacks targeting and delivery specificity and remains time dependent. By utilizing a direct current of relatively low amplitude, an active electrode is placed on the drug formulation that is to be driven into the skin by the electrons streaming from the active electrode. The ionic charge imparted to the target molecule allows the molecule to be driven into the skin as the indifferent ions are pulled from the skin by the indifferent electrode to complete the circuit. This process has typically been used to drive small charged molecules across skin. Interestingly, iontophoresis was reported to be successful with botulinum toxin for treating palmar hyperhidrosis although the practice has not become widely adopted since the initial reports (see Kavanagh et al and Solomon). The disadvantages are that the technique is very time and concentration dependent, requires that the patient have the ability to tolerate the sensation of the direct current, and is less effective with delivery of lipophilic molecules.

Beyond iontophoretic strategies, a number of new investigational approaches to topical delivery of therapeutics have evolved and several are being evaluated for BoNT-A. These approaches rely on refinements of conventional topical drug delivery strategies including liposomes as well as developments in molecular biology including the past decades of progress in cell-penetrating peptides (CPP, also known as protein transduction domains or PTDs; see Chaichir et al). A CPP-based approach for transcutaneous delivery of BoNT-A is RT001 Topical Gel (Revasive Therapeutics Inc.). The safety and effectiveness of this approach have been previously reported from initial studies in both axillary hyperhidrosis and LCL by Glogau and by Brandt et al. Chaichir et al had previously reported on microcrystals and ionic nanoparticles for topical BoNT-A in a daily application (DPM Therapeutics Corp./Transdermal Corporation). The magnitude of the results from the nanoparticle study was surprising given that the outcome was achieved with daily topical application of a BoNT-A dose that would not be expected to attain efficacy when injected. Recently, clinical trials have also been initiated to evaluate the safety and effectiveness of liposomes with a controlled size range to deliver BoNT-A for axillary hyperhidrosis and LCL (Anterios Inc.).

Although neither Transdermal Corporation nor Anterios are BoNT-A manufacturers, both have described long-term stability of BoNT-A in their topical preparations. Neither intends to require reconstitution of a lyophilized BoNT-A at the time of use. Interestingly, no company that manufactures BoNT-A has been able to develop a solution stable form of BoNT-A commercially. Since all current manufacturers of BoNT-A including all injectable forms [Allergan’s Botox® Cosmetic (onabotulinum toxin A); Ipsen’s Dysport® (abobotulinum toxin A); Xeomin® (incobotulinumtoxinA) from Merz GmbH; Purex® from Mentor/J&J in USA; Neuronox® from Medy-tox in Korea; and Prosigne®/Hengli® from Lanzhou Institute in China] as well as the investigational topical product RT001 (Revasive Therapeutics in USA) require reconstitution of a stabilized lyophilized BoNT-A, this may prove a key point for Transdermal Corporation and Anterios.

The remainder of this section will detail progress on RT001, which is the furthest along in clinical development of an investigational topical BoNT-A preparation. RT001 is a topical BoNT-A comprised of the 150 kilodalton (kDa) pure neurotoxin, which is ionically paired with a CPP-based peptide to afford transdermal penetration. There are two distinct yet complementary pathways that facilitate transcutaneous delivery using a TAT-based CPP such as that in RT001. The first mechanism is lipid rafting, which is energy independent and occurs on the non-living cells of the stratum corneum. The second mechanism is energy dependent and occurs only across living cells; this pathway is a transcytosis shuttle, which is a variant of induced macropinocytosis. This mechanism is the rate-limiting step for delivery of RT001 so thinning or removing the stratum corneum appears to have no impact on drug delivery of this BoNT-A. For macropinocytosis, the CPP induces the cell to take a large ‘drink’ of the surrounding including the CPP. Unlike simple macropinocytosis, this variant pathway then releases the contents on either side of the cell. When returned to the original side no net change occurs, but when returned to the opposite side the contents have crossed the cell. The result is a net flux along the concentration gradient across the cells. This pathway likely takes place on living epithelial cells lining glands and deeper skin cells, and manifests as a net directional flux from apical to basolateral surface.

To self-assemble a particle containing both a therapeutic active like botulinum toxin and a CPP-containing carrier, some form of non-covalent bond must be employed. For biologically active proteins like BoNT-A, which often have a conserved motif of high densities of negatively charged amino acids on their surface, ionic bonding is particularly facile. RT001 relies on these ionic interactions to assemble the CPP-containing peptides on the surface of BoNT-A.

Based upon the initial reports of RT001 by Glogau and Brandt et al, as well as prior unpublished work, a full Phase II clinical program for the temporary improvement in the appearance of moderate to severe lateral canthal lines was completed including over 550 subjects exposed to RT001. Dose escalation studies were undertaken across five studies for RT001 in LCL. These studies
Table 10.1  Response rates by 2 points or greater improvement on IGA-LCL 4 weeks after treatment with RT001

<table>
<thead>
<tr>
<th>Dosage of BoNT-A</th>
<th>All controls n = 200</th>
<th>RT001 n = 13</th>
<th>RT001 n = 11</th>
<th>RT001 n = 38</th>
<th>RT001 n = 61</th>
<th>RT001 n = 136</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.3 ng/mL</td>
<td>5.5 ng/mL</td>
<td>11 ng/mL</td>
<td>22 ng/mL</td>
<td>25 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Percentage of subjects responding in both eyes</td>
<td>8.5%</td>
<td>7.7%</td>
<td>18.2%</td>
<td>22.2%</td>
<td>29.5%</td>
<td>51.5%</td>
</tr>
<tr>
<td></td>
<td>p = 1.0000 [b]</td>
<td>p = 0.2592 [b]</td>
<td>p = 0.0334 [b]</td>
<td>p &lt; 0.0001 [a]</td>
<td>p &lt; 0.0001 [a]</td>
<td></td>
</tr>
<tr>
<td>Percentage of LCA responding (n = twice the number of subjects listed)</td>
<td>9.8%</td>
<td>7.7%</td>
<td>18.2%</td>
<td>28.4%</td>
<td>34.4%</td>
<td>56.3%</td>
</tr>
<tr>
<td></td>
<td>p = 1.0000 [b]</td>
<td>p = 0.2637 [b]</td>
<td>p &lt; 0.0001 [a]</td>
<td>p &lt; 0.0001 [a]</td>
<td>p &lt; 0.0001 [a]</td>
<td></td>
</tr>
</tbody>
</table>

Note: The p-value is for the pair-wise comparison of RT001 versus placebo using [a] Pearson's chi-square test or [b] Fisher's exact test.

Figure 10.1  A representative 2-point mover in the Phase II trials: (A) baseline; (B) 4 weeks post-treatment. Reproduced with permission of Revance Therapeutics, Inc.

(1) encompassed all experiences with the proposed Phase III dose of peptide excipient, (2) employed similar efficacy scales, enrollment criteria, formulation, timepoints, and consistency of treatment effect, and (3) reflected planned Phase III studies. All studies that were similar have been included in this analysis. Escalating doses of RT001 from 5.5 to 25 ng/mL of BoNT-A led to an increasing proportion of responders based on 2-point or greater improvement on a validated investigator global assessment of lateral canthal line severity (IGA-LCL scale) whether viewed by individual lateral canthal area (LCA) per protocol or subject (both eyes responding as proposed for Phase III studies) as detailed in Table 10.1. An example is shown in Figure 10.1.

In regard to safety, none of these clinical studies regardless of concentration combination revealed any safety signals of clinical relevance. Specifically, the efficacy increases observed with escalating doses of RT001 were not accompanied by dose-dependent increases in adverse events. Glogau et al. reported treatment-emergent adverse events were generally mild and transient, as in the previously reported studies by Brandt et al. Local effects included neuromuscular events directly related to RT001 action on its target muscle, as well as generally mild and transient eye and skin events that were not dose related. Safety assessments of cranial nerve evaluation, skin and ocular irritation, clinical laboratories tests, and ECG showed no significant treatment or dose related findings. No evidence of diffusion to adjacent muscles has been seen with RT001. No evidence of systemic spread or systemic safety issues was observed in any study. There were no treatment-related increases in antibody titers to the neurotoxin or the peptide compared with pre-dose serum samples. Given this safety profile, reducing the dose of either RT001 component or both components could not improve the product's overall safety profile and reducing the concentrations of one or both components would likely decrease efficacy. Any dose reduction would
likely have no effect on the combination product's risk profile, but would have a negative effect on the combination product's potential benefit. The dose selected for further clinical development is the lowest dose of each to achieve adequate efficacy with adequate safety.

After dosage selection, efficacy of the proposed Phase III dose of RT001 was demonstrated in two confirmatory Phase II studies by Globau et al. In these studies RT001 Topical Gel demonstrated efficacy following a 30-minute application in comparison with controls for the treatment of subjects with moderate to severe lateral canthal lines related to orbicularis oculi muscle spasm, as summarized in Table 10.2. End points employed both investigators' assessments (IGA-LCL) and subject assessments (PSA) of lateral canthal line severity as well as composites of both measures. Additionally subject self-perception of improvement was tabulated. The 25 ng/mL dose of RT001 demonstrated statistically significant efficacy versus placebo on the primary composite endpoint as well as all secondary endpoints and was well tolerated.

### Table 10.2 Efficacy analyses for the proposed Phase III dose of RT001

<table>
<thead>
<tr>
<th>Subjects assessed at week 4 (N)</th>
<th>RT001 subjects (25 ng)</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite ≥2-point improvement in PSA and ≥2-point IGA-LCL improvement in both LCA</td>
<td>41.9% ( p &lt; 0.0001 )</td>
<td>0.74%</td>
</tr>
<tr>
<td>≥2-Point IGA-LCL improvement in both LCA</td>
<td>51.5% ( p &lt; 0.0001 )</td>
<td>10.6%</td>
</tr>
<tr>
<td>≥2-Point improvement in PSA</td>
<td>46.3% ( p &lt; 0.0001 )</td>
<td>3.0%</td>
</tr>
<tr>
<td>≥1-Point IGA-LCL improvement in both LCA</td>
<td>75.7% ( p &lt; 0.0001 )</td>
<td>22.0%</td>
</tr>
<tr>
<td>PGIC improved or much improved</td>
<td>52.9% ( p &lt; 0.0001 )</td>
<td>7.6%</td>
</tr>
</tbody>
</table>

Note: The *p*-value is from the Pearson's chi-square test.

### Further reading

- Chajchir I, Modi P, Chajchir A 2008 Novel topical BoNTA (Cosme'RoX, Toxin Type A) cream used to treat hyperfunctional wrinkles of the face, mouth, and neck. Aesthetic Plastic Surgery 32:715–722
- Globau RG 2007 Topically applied botulinum toxin type A for the treatment of primary axillary hyperhidrosis: results of a randomized, blinded, vehicle-controlled study. Dermatologic Surgery 33:S76–S80

Matarasso SL, Matarasso A 2001 Treatment guidelines for botulinum toxin type A for the periorcular region and a report on partial upper lid ptosis following injections to the lateral canthal rhytids. Plastic and Reconstructive Surgery 10:208–214


