

RESEARCH LETTERS

A Small Study of the Relationship Between Abobotulinum Toxin A Concentration and Forehead Wrinkle Reduction

Botulinum toxin A has been used in the cosmetic treatment of facial rhytids for decades. Previous studies have examined the role of volume and concentration on the spread of onabotulinum toxin A, both on and off the face, and on the treatment of dynamic rhytids¹ and muscle spasticity.² Our clinical experience suggests that abobotulinum toxin A may in fact spread differently than its counterparts. To determine if this was true, we treated 10 patients with each of 2 concentrations of abobotulinum toxin A to assess differences in rhytid reduction on contralateral sides of the forehead. We hypothesized that dilution may affect capacity of the toxin to spread to neuromuscular receptors, which may in turn affect surface area of effect.

Methods. After the research protocol was approved by the New England institutional review board, 10 patients, aged 35 to 65 years, with moderately severe forehead rhytids (Glogau II and III)³ were recruited to participate in a study involving the delivery of 6 U of abobotulinum toxin A (Dysport; Medicis) to the right and left forehead. On one side, 6 U were diluted in 0.1 mL of sterile preserved saline solution. On the other side, 6 U were diluted in 0.3 mL of sterile preserved saline solution. The product was diluted in a 20-mL syringe after extraction from its original bottle to achieve the dilutions required by the study protocol.

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Subjects were randomized with regard to who received which concentration on a particular side, and on both sides the product was delivered to prominent horizontal rhytids measuring between 2.5 and 3.0 cm above the orbital rim in the mid-pupillary line (**Figure 1**). Abobotulinum toxin A was injected with minimal pressure at a 90° angle of incidence to a depth of approximately 1 mm below the surface of the skin. Exclusion criteria included pregnancy; history of botulinum toxin injection to the forehead in the prior 12 months or long-acting dermal filler injection (including silicon, Sculptra [sanofi-aventis], and Radiesse [Merz]) to the forehead at any time; history of neuromuscular disease or palsy; prior hypersensitivity to a botulinum toxin; and concomitant use of medications affecting neuromuscular blockade including aminoglycosides, anticholinesterases, lincosamides, and polymyxins.

All patients were photographed at rest and at maximum brow elevation, and distances from the orbital rim

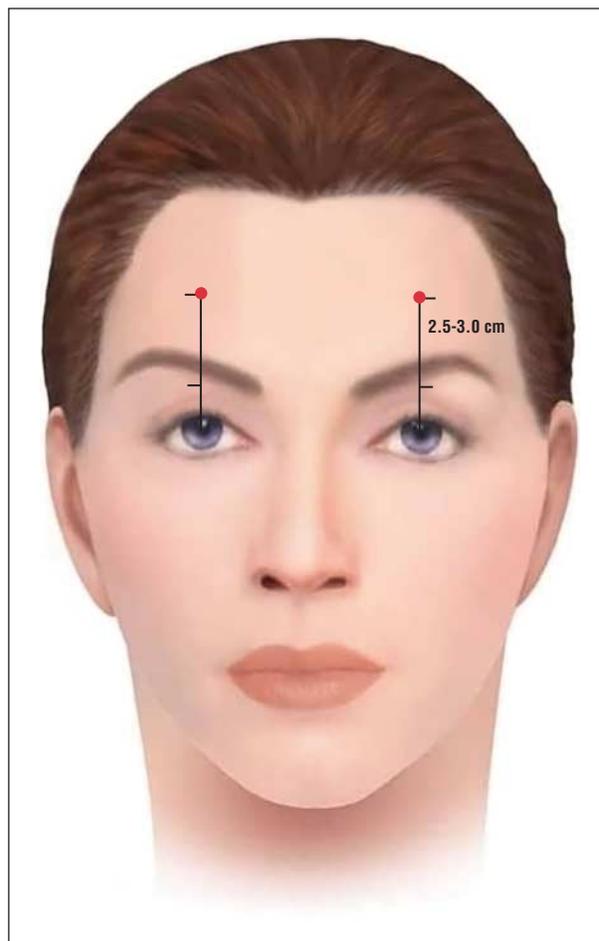


Figure 1. Abobotulinum toxin A injections were placed in the first prominent horizontal crease located between 2.5 and 3.0 cm above the orbital rim. Orbital rim measurement is independent of brow location, which is variable and should not be used in determining injection sites in both clinical and research settings.

to the injection site were carefully measured. The same unblinded physician (N.R.A.) treated and photographed all subjects, while a separate, blinded physician (K.A.A.) recorded rhytid reduction on actual subjects (ie, not photographs) at 14 days post treatment. The blinded physician marked the horizontal and vertical extent of wrinkle reduction in millimeters around the original injection site with a red pen. Subsequently, wrinkle reduction was measured in millimeters squared on each side of the forehead, and the 2 treated sides were compared.

Results. Nine men and 1 woman between the ages of 35 and 63 years were recruited. All participants experienced visible rhytid reduction to both sides of the forehead, and none had adverse reactions including bruising, headache, and eyebrow or eyelid ptosis.

Statistical analysis of rhytid reduction revealed a marked and statistically significant difference between

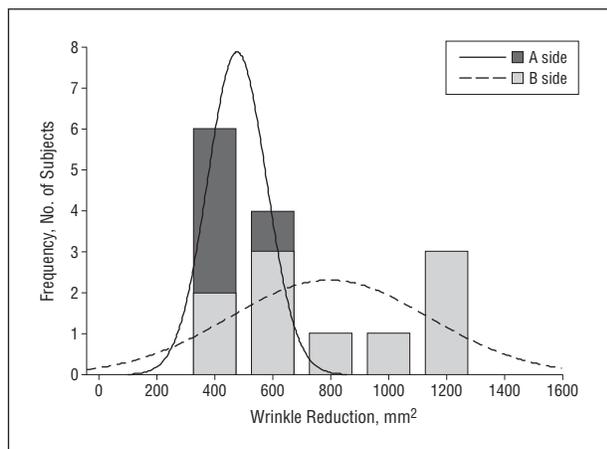


Figure 2. Wrinkle reduction response data are depicted using the histogram by treatment side, an effective graph for portraying dispersion and central tendency. The A side is the more concentrated and the B side the more dilute preparation of abobotulinum toxin A. Under the assumption of normality, which was confirmed via the Anderson-Darling test, both the conventional *t* test (A vs B means) and the paired difference *t* test (A-B differences by subject) were applied to test the response in wrinkle reduction. Both *t* test results were statistically significant with greater than 98% and 99% confidence, respectively, confirming that B-side treatment resulted in a higher mean (SD) reduction in wrinkles: 794.1 (345.6) mm² vs 476.6 (101.3) mm².

contralateral sides of the forehead vis-à-vis abobotulinum toxin A concentration. Consistently, the more dilute concentration of the product produced a larger surface area of rhytid reduction. The more concentrated solution produced a mean wrinkle reduction of 476.6 mm², while the more dilute solution produced a mean wrinkle reduction of 794.1 mm² (**Figure 2**). *P* values for both data sets supported statistical significance. Paired *t* tests and box plot analyses confirmed these findings (data not shown).

Photographs revealed that the pattern of wrinkle reduction in most subjects did not exhibit a perfectly circular pattern but rather varied from ovoid to irregular, with some patients clearly exhibiting more wrinkle reduction on one side of the injection site.

Comment. There is no standard conversion ratio for comparing different formulations of botulinum toxins, making the comparison of one type with another difficult.⁴ Moreover, there is no consensus among practitioners whether low-volume/high-concentration injections of botulinum toxins or higher-volume/lower-concentration injections produce better clinical outcomes.⁵ While there are data to support the value of lower-concentration/“more spread” techniques in particular muscle groups including the frontalis muscle,¹ other studies report no advantage to variations in concentration of onabotulinum toxin A when treating periorcular rhytids⁵ or muscle spasticity.² Abobotulinum toxin A diffusion has been little studied owing to its more recent emergence into clinical practice.

In certain clinical situations, such as in the injection of glabellar muscles, clinicians may be rightly concerned about unwanted spread producing complications including muscle ptosis. In the injection of larger or broader muscle complexes, including the frontalis, the

concept of spread may prove advantageous to clinical outcome. The variation in spread across patients in a small study such as this one makes generalizations about product spread across a larger population difficult. However, our findings reinforce the principle that product diffusion is a complex phenomenon likely influenced by factors including bulk of muscle mass, dynamic movement of muscles, and actual dose delivered.

This study suggests that dilution of abobotulinum toxin A may help to improve delivery to a wider surface area and thus may allow clinicians to achieve desired clinical outcomes while using modest doses of toxin. Longevity of rhytid reduction on each side of the forehead was not assessed because the treatment resulted in marked asymmetry of rhytids at 2-week follow-up that required correction with additional product. Future studies to assess the relationship between product dilution and longevity would be useful, as would specific comparisons of the diffusion properties of different botulinum toxins used in medical practice.

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PRACTICE GAPS

Dilution, Reconstitution, and Complexity

The treatment of dynamic upper facial creases with botulinum toxin type A is sometimes characterized as a trivial procedure, one equally successful in the hands of anyone capable of holding a syringe and depressing a plunger. In fact, this procedure is surprisingly complex and replete with nuance. With this study, Abbasi et al further define the properties of different dilutions of botulinum toxin.

The specific practice gap addressed is how dilution affects effectiveness, and whether effectiveness and safety can be improved by using more dilute botulinum toxin preparations. However, in a larger context, the practice gap is not merely uncertainty about dilution, but more generally, how botulinum toxin should be deployed for cosmetic treatments. When botulinum toxin was classified by the US Food and Drug Administration as a drug, the subtleties of its use were not yet very well understood, and official guidance about dosage and administration on the package insert is consequently sparse. In practice, botulinum toxin operates with the site-specific concentration and means of delivery more important than total dose. Furthermore, as the number of routine off-label indications like forehead, crow's feet, and lower face wrinkles has grown, the question of where, what, and how to inject has only become more convoluted.

Dilution is itself an inaccurate term, as *reconstitution* better describes combining solid prepackaged toxin with fluid. Among the substances that have been used to reconstitute botulinum toxin are normal saline, normal saline with the preservative benzyl alcohol,¹ and lidocaine.² The last two have been shown to markedly diminish injection pain without notable loss of effectiveness. Another variable is concentration, with fluid volumes of 1 to 10 mL routinely used to reconstitute an entire vial of botulinum toxin. For onabotulinum toxin A, equipotent injections with the resulting range of concentrations have been shown not to affect

trained-observer ratings of treated glabellar³ and crow's feet⁴ wrinkles. This is obviously in contradistinction to the study presented by Abbasi et al, which focused on the forehead and assessed the utility of abobotulinum toxin A.

So the specific formulation of the type A toxin is yet another variable. As the authors note, there is no strict conversion between the units of onabotulinum and abobotulinum toxins, but some have suggested that the ratio is somewhere between 1.0:2.5 and 1:3. Equipotent injections of these 2 toxins reconstituted in the same amount of fluid have been reported to have marginally different effects, with the radius of effect (ie, *action halo*) of the abobotulinum toxin on the forehead slightly greater than that of onabotulinum toxin.

Thus, another variable is the exact muscle group injected, with the commonly injected glabella, forehead, and crow's feet sites not necessarily responding symmetrically to changes in dilution and toxin subtype. Depth of injection may be a further functional variable, and this can be intradermal or at the periosteum. Force and speed of injection may impact the distance of physical dispersion of the injectate, with dilution held constant. It has been shown that botulinum toxin can be stored for weeks to months after reconstitution,⁵ and that the same vial can be reaccessed for multiple treatments without contamination⁶; so storage, surprisingly, does not appear to be an important variable.

The results reported by Abbasi et al are particularly exciting because they suggest that similar effects can be obtained with smaller, less expensive, and presumably less toxic (no pun intended) doses. The authors did not report increased injection pain in the low-concentration group, as has been reported by Boyle et al⁷ in the ophthalmic literature, and this absence of pain may have been intrinsic or attributable to the very small injection volumes used. Similarly, the findings of Abbasi et al may be particular to the face, since higher volume injections have not been found to be more effective in limb spasticity applications.^{8,9}

In summary, a series of small, inexpensive studies can answer important clinical questions. And even seemingly simple cosmetic procedures are like fractal patterns, which become progressively more complex the more closely they are studied (**Figure**). This is probably why experienced injectors are loathe to change their technique because minor tweaks may result in nonlinear, unpredictable changes in results.

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Fluid Used	Total Volume	Toxin Type	Muscle	Depth	Speed
Saline with preservative	1 mL	Onabotulinum	Frontalis	Dermis	Fast
Saline without preservative	2.5 mL	Abobotulinum	Orbicularis	Periosteum	Slow
2 ¹ ⇒ 2 ² ⇒ 2 ³ ⇒ 2 ⁴ ⇒ 2 ⁵ ⇒ 2 ⁶					

Figure. Assuming that each of the known variables that may affect botulinum toxin effectiveness for facial wrinkles has just 2 possible values, there are at least 2⁶, or 64, permutations.