**Effect of Volume and Concentration on the Diffusion of Botulinum Exotoxin A**

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**Objective:** To investigate whether the volume of solution used to inject equivalent units of botulinum exotoxin A affects the diffusion of toxin and areas of rhytid diminution in the treatment of dynamic forehead lines.

**Design:** Ten volunteers with dynamic forehead lines were included. Each study patient received a single injection at a point 2.5 cm above the orbital rim on either side of the forehead with equivalent units, but in different volumes, of botulinum exotoxin A. The sides of injection were randomized; one side of the patient’s forehead was injected first with 5 U of botulinum exotoxin A in 0.25 mL (2 U/0.1 mL) of preserved saline in the midpupillary line, followed by injection of the other side with 5 U in 0.05 mL of preserved saline (2 U/0.02 mL). There was a 5-fold difference in volume injected. Subjects were evaluated 14 days later for total area affected during visual inspection of the subjects’ foreheads during active muscle contraction.

**Setting:** Private dermatology office.

**Main Outcome Measure:** Visual inspection to measure the area of rhytid effacement in both height and width.

**Results:** The area affected by the botulinum exotoxin A injection was 50% greater in the side with the larger volume in 9 of 10 subjects. The average area affected was 6.05 cm² for the injection of the larger volume compared with 4.12 cm² for the injection with the smaller volume. The shape of rhytid effacement was oval, rather than round, with the average width longer than the average height.

**Conclusions:** In this prospective, randomized, controlled study, we found that injection of botulinum exotoxin A in low concentration and higher volume resulted in greater diffusion and a larger affected area. The pattern of toxin spread is altered by muscular contraction in the injected sites. These results show that the dilution has implications on the desired effect of botulinum exotoxin A.

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**METHODS**

There are great variations among physicians in the amount of saline used to reconstitute a standard bottle of botulinum exotoxin A (Botox Cosmetic; Allergan Pharmaceuticals, Irvine, Calif), with studies advocating dilutions ranging from 10 U/0.1 mL to 1 U/0.1 mL.** It** Despite the great variation in opinions regarding the best dilution and volume of injection for cosmetic applications, investigators have focused primarily on efficacy and duration of action rather than on volume and dilution. Therefore, we investigated whether the volume of solution used to inject equivalent units of botulinum exotoxin A results in a difference in area of effect and diffusion of toxin in the treatment of dynamic forehead rhytids.

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Ten healthy adult volunteers with dynamic forehead lines were included in our prospective randomized controlled study. The forehead was chosen as the treatment site because it allows better visualization of the horizontal dynamic rhytides and centrifugal spread of the toxin’s effect. Inclusion and exclusion criteria were strictly followed (Table 1). Each study patient received a single injection on either side of the forehead with equivalent units, but in different volumes, of botulinum exotoxin A. The sides of injection were randomized; one side of the patient’s forehead was injected first with 5 U of botulinum exotoxin A in 0.25 mL (2 U/0.1 mL).
Botulinum exotoxin A is a potent neurotoxin that inhibits the release of acetylcholine. The resultant decrease in transmission of neural impulses results in weakened contraction of the underlying muscles and reduction of rhytids. The precise placement and appropriate dose of botulinum exotoxin A injection are key to optimizing the outcome. One of the considerations for appropriate dosing is the volume of dilution, which has varied widely among practitioners.

Each vial of botulinum exotoxin A is freeze-dried, containing 100 U of toxin. Most clinicians use between 1.0 and 3.0 mL of saline for dilution, but some report using dilutions of up to 10 mL. Some believe that a small amount of solution (higher concentration) is difficult to work with, ultimately leading to waste of material. Yet others have speculated and reported anecdotally that a higher dilution (lower concentration) encourages the spread of the toxin, too much of which may be undesir-

Table 2 gives the width, height, and area of effect from the 2 dilutions. In 9 of 10 subjects, the side injected with the larger volume produced a larger area of effect. The injections produced the same area of effect in the 1 remaining subject. For the side injected with the smaller volume (2 U/0.02 mL), the mean width of the area of rhytids effacement was 2.28 cm (range, 1.6-2.6 cm), and the mean height of effacement was 1.91 cm (range, 1.5-2.9 cm). The mean area of effect was 4.12 cm² (width × height). For the side injected with the larger volume (2 U/0.1 mL), the mean width of the area of rhytids effacement was 2.71 cm (range, 1.9-3.3 cm), and the mean height of effacement was 2.23 cm (range, 1.7-2.6 cm). The mean area of effect was 6.05 cm². These differences are statistically significant using a 2-tailed t test (P = .003). Figure 1 and Figure 2 show 2 examples of the difference in the size of the area affected by the different volumes.
With a higher volume, the areas of diffusion appear to increase, although until the present study, no controlled human studies had verified this observation. The ideal strategy may be to inject concentrated toxin at a low volume to target smaller muscle groups, while using a larger volume for larger, broad muscle groups, such as the frontalis muscle. One strategy for effectively handling a smaller volume of injection without waste is to use a proper syringe. To ensure precise delivery of the toxin, we used a short-needle, 30-gauge, 0.3-mL insulin syringe (Becton Dickinson), which allows the delivery of a precise number of units, even in half-unit doses. The needle design allows no dead space in the needle hub. Full depression of the plunger leaves less than 0.01 mL in the needle itself, a contrast with the 0.07 mL that is retained in the dead space in a traditional 30-gauge needle. At 100 U/mL, this means that 7 U of botulinum exotoxin A cannot be used. The syringe comes with a silicone-coated 30-gauge needle, which easily penetrates the skin. The needle stays sharp for approximately 4 to 6 punctures.

Although greater volume leads to more diffusion into surrounding tissue, a possible consequence may be diminution in duration and magnitude. This issue was not addressed in our study. The assumption has been that the higher the concentration, the longer the duration of action. Indeed, some practitioners have found that the results from these large dilutions are short-lived, and perhaps only weaken but do not completely eliminate the contraction of the target muscles. However, there are no large studies to support this hypothesis. Also, the increased diffusion can potentially affect unintended muscle groups. For example, the unwitting physician may inject too close to the eyelid, resulting in cosmetically devastating, although short-lived, eyelid ptosis.

A study by Kim et al on a rabbit model revealed surprising and counterintuitive results regarding dose.
evaluated the effectiveness of botulinum exotoxin A dilution volume by measuring the compound muscle action potential (CMAP) amplitude to indicate levels of muscle paralysis. After injection with the same number of units but in different dilutions, there was a significant decrease in CMAP amplitudes in the 10 U/0.5 mL group compared with the 10 U/0.1 mL group at 1 week and at 4 weeks. So, paradoxically, the larger-volume, lower-concentration group led to greater paralysis. Kim and colleagues’ findings were supported by those of Shaari and Sanders,6 who found that at a constant dose of 0.2 U, increasing the volume of injection resulted in increased paralysis. However, Shin et al7 measured no significant difference in CMAP amplitude between dilutions of 2.5 U/0.1 mL and 2.5 U/0.5 mL injected into human muscle. Similarly, Francisco et al8 compared the efficacy of 2 different-volume preparations of the same dose of botulinum exotoxin A in relieving spasticity in wrist and finger flexors, but saw no significant spasticity reduction between the high- and low-volume groups. This difference might be a result of the total dose of the toxin injected. If a relatively small muscle is injected, and if the dose of toxin is sufficient to paralyze the muscle, increasing the dilution volume would have little additional effect on the muscle. It would be worthwhile to repeat our experiment with long-term follow-up to observe the difference in duration of action, if any.

It must be emphasized that the present study involved only the forehead, and the results and conclusions may only be applicable to the forehead. In contrast to our findings, Carruthers et al9 found no difference in the rate of response, degree of improvement, or the rate of relapse when varying the dilution of a 30-U dose of botulinum exotoxin A to the glabella. The difference in injection into a single muscle group in the forehead (the frontalis muscle) and injection into multiple muscle groups in the glabella (corrugator, procerus, orbicularis oculi, and depressor supercilii muscles) might account for such different observations.

A possible criticism of our study is the method of measurement. Visual measurement of area of rhytides effacement may be imprecise. A more objective measurement would be electrophysiologic measurement using CMAP, as described above. Other investigators have reported increased accuracy using the starch-iodine test to measure area of anhidrosis after botulinum exotoxin A injections. The obvious visual cue from the test may enhance the accuracy of measurement (D. M. Hextel, MD, oral communication, October 10, 2003). Revealing only 1 side of the individual forehead during measurement may also decrease the possible bias that might arise when the 2 sides are compared side by side. Some clinicians may take issue with our estimating the area of effect by simply multiplying the height and width. However, we found that although the toxin spreads in a centrifugal fashion, it appears to produce a more ovoid than circular area, with the average width longer than the height. This phenomenon may be the result of the vertical contraction of the frontalis muscle pushing the injected material horizontally, especially with the forehead exercise suggested immediately after the injection. We believe that rather than calculating the area of a circle, it is simpler and probably more accurate to quantify the area by multiplying the height and the width.

With the same number of units, injection using higher volume results in greater diffusion and a larger affected area. In the present study, we saw an approximate 50% increase in area simply by increasing the volume 5-fold. The results indicate that we can add another level of sophistication to the use of botulinum exotoxin A by varying the level of precision. To treat larger, confluent areas, such as the forehead, a larger volume can be used to achieve more spread. This also means fewer injections, which is important in the pain-averse patients. Conversely, smaller volumes can be used to treat functionally sensitive areas, eg, around the eyes or the mouth, to avoid excessive diffusion of the toxin. The physician must also recognize that the pattern of muscular contraction appears to alter the direction of toxin diffusion, so it is not possible to predict the shape of the resultant centrifugal spread. It is clear, however, that the toxin does not spread in an even annular fashion into a target or “bull’s-eye” pattern.

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