Incorrect Reconstitution of IncobotulinumtoxinA Leads to Loss of Neurotoxin

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ABSTRACT

Background: IncobotulinumtoxinA (INCO) was approved for aesthetic use in the United States in 2011. When reconstituted per manufacturer’s instructions, diminished delivery of INCO in US may result.

Objective: Investigators sought to determine whether potential loss of decreased motor activity could be demonstrated, using a simple reconstitution technique.

Methods and Materials: In this 5-patient study, investigators added 2.0 mL of saline to INCO powder at the bottom of the first of 5 vials, swirling gently to dissolve INCO powder at the bottom. Reconstituted INCO was discarded and the cap was replaced. The “empty” vial then received 0.6 mL of saline, and was swirled and inverted 3 times to ensure dissolution. The 0.6 mL from the first vial was added to the second “empty” vial and the process was repeated for the remaining 3 vials (5 vials per patient). Patients were injected from reconstitution of “empty” vials to determine neuromodulatory activity. Pre- and post-treatment patient photographs of maximal contraction were taken.

Results: Markedly diminished maximal frown could be observed in all 5 patients.

Conclusion: Improper reconstitution of INCO, or swirling without inversion of the vial following saline injection, can result in significant loss of units of the neurotoxin in the clinical setting.


INTRODUCTION

IncobotulinumtoxinA (INCO; Xeomin® in the United States, Canada / "botulinum toxin A free of complexing proteins" or Bocouture® in Europe and other parts of the world) is distributed by Merz Aesthetics Inc. and Merz Pharmaceuticals LLC in Greensboro, NC. INCO has been in approved use in Europe since 2005, with availability in 20 countries and with over 261,000 patients treated.1

Recently, anecdotal reports from North American aesthetic clinicians have suggested some variance in efficacy amongst BoNTA products2 despite many studies demonstrating comparable effectiveness of toxins when dosed properly. Studies of more than 2000 patients have shown comparable dosing, efficacy, and safety comparing INCO to onabotulinumtoxinA (ONA; Botox®, Allergan, Irvine, CA).3-5 These anecdotal reports raise the issue of variables, if any, which may be associated with differences in efficacy.

One difference may be related to how INCO is supplied in Europe, compared to North America. In Europe, INCO appears as a compressed “puck” at the bottom of its vial. In North America, INCO may appear as a puck with most of the neuromodulator at the bottom of the vial. However, it is often present as a loose powder, clustered around and inside the rubber cap, as well as on the sides of the vial, possibly due to product shifting during overseas transport (Figure 1).6 We wondered whether physical differences in INCO presentation in its vial would influence reconstitution technique, and thus account for the anecdotal comments upon differences in efficacy.

Our hypothesis is that, because INCO can be found distributed throughout the vial rather than solely at the bottom of the vial, failure to invert a vial of INCO during the addition of saline results in reconstitution of less than 100 percent of the available neuromodulator, possibly resulting in diminished efficacy upon injection.

The idea of incomplete reconstitution arose from an inadvertent swirl and inversion of vials of INCO by the author, rather than the usual simple swirl reconstitution technique as traditionally used for ONA. The author typically uses a 2 cc dilution of INCO or ONA. This technique came about when we noticed that there was no foaming of the ONA solution when saline was injected into the inverted vial. This technique was continued when we started reusing INCO. When injected into the patient, the INCO reconstituted from a swirled and inverted vial resulted in a clinically equivalent effectiveness of neuromuscular paralysis as compared with ONA injections in our practice. This serendipitous inversion led us to design an experiment to determine if, by simply altering the reconstitution technique of INCO, optimization of the clinical effect of decreased motor activity could be demonstrated.

Materials and Methods

Five patients who were naïve to BoNTA injections were selected for this trial. Vials of INCO were included in the study only if there was visible powder around and inside the vial cap. Vials that contained INCO in complete or slightly disrupted puck form were not included in the study (Figure 1).

Reconstitution Method

Step 1. Five vials of INCO were each reconstituted with 2.0 mL of sterile, preserved 0.9% sodium chloride (saline). The vials were
FIGURE 1. IncobotulinumtoxinA may appear as either a “puck” (upper image) or a powder (lower two images). As a powder, most of the incobotulinumtoxinA may be found at the bottom of the vial but may also be clustered around and inside the cap of the vial as well as on the walls of the vial.

Swirled gently, dissolving only the neurotoxin at the bottom of the vial. The cap was gently removed, so as not to disturb any powder residue on the side or bottom of the cap, and the 2 mL of reconstituted INCO was withdrawn and discarded. The cap was replaced on each vial. (Figure 2)

Step 2. The investigator added 0.6 mL of saline to the first “empty” vial, then swirled and inverted three times to ensure incorporation of all residual powder. (Figure 3)

Step 3. The 0.6 mL of saline was then withdrawn from the first vial and added to the second “empty” vial, swirled and inverted 3 times, and the process repeated sequentially for all 5 vials. The pooled saline of all 5 vials, containing an unknown number of possible residual units of INCO, in a total of 0.6 mL of saline, were then used to inject one patient, and to assess its neuromodulatory effect. This process was repeated for all 5 patients in the study for a total of 25 vials. (Figure 4)

Later, this reconstitution process was also conducted, pooling the residue of only 3 vials, and then reconstituting only 1 vial, in order to better estimate how much unreconstituted toxin powder remained on the cap and the sides of the vial. (Figure 4)

Injection Method
To determine whether any neuromodulatory activity was present in the pooled saline, the 5 Caucasian female patients were each injected with INCO from the reconstitution of 5 vials as described above, and then seen at 2- and 4-week follow-up visits. In addition, 1 patient was injected with reconstituted product pooled from 3 vials, and another from the reconstitution from a single vial. The age range was from 21 years to 55 years. The sites of injection included the procerus muscle, the bilateral heads of the corrugator muscles, and the bilateral tails of the corrugator muscle. (Figure 5)

A total volume of 0.5 mL of pooled saline was injected per patient. A 0.1 mL loss during multiple reconstitutions was factored into the final volume injected. The injection pattern is detailed in figure 5.
Control experiments were conducted in 5 additional patients. The initial step after instilling 2 mL of saline was followed by inversion and rotation of the vial 3 times, instead of simply a gentle swirl. For each control patient, the same reconstitution method was used as described above, pooling the residual of 5 vials in 0.6 mL of saline. Each patient was then injected with 0.5 mL of the inverted and pooled solution.

RESULTS

Clinical effect could be observed in all patients: the 5 patients injected with the pooled residue of 5 vials (Figure 6), in the 1 patient injected with the pooling of 3 vials, and the 1 patient injected with the residue of 1 vial (Figure 7), with comparable degrees of potency. All patients experienced markedly diminished ability for maximal frown. Table 1 is a summary of the results obtained using the 5-point Merz Aesthetics Scales for glabellar lines at rest and in dynamic phase.16

DISCUSSION

This experiment was intended to demonstrate that improper reconstitution of INCO, or swirling without inversion of the vial following saline injection, can result in significant loss of...
TABLE 1.
Score on the Merz Aesthetics Scale for Dynamic Glabellar Lines in 5 Patients at Baseline, 2 Weeks, and 4 Weeks Post Injection

<table>
<thead>
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<th>Patient</th>
<th>Merz Aesthetics Score</th>
<th>2 weeks post treatment</th>
<th>4 weeks post treatment</th>
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units of the neurotoxin in the clinical setting. As Figures 6 and 7 demonstrate in this experiment, we show that the injection of pooled residuals from “empty” vials (1, 3, or 5 vials) obtained a significant and sustained clinical effect, and was not simply a placebo, as one would expect if the injection were 0.5 mL of saline alone. Furthermore, injection of the residual saline from properly reconstituted vials shows that there are no remnants of toxin left on the cap and on the sides of the vial through inversion and rotation 3 times during the mixing process.

The most efficient manner to ensure that all INCO in powder form is reconstituted to 1) completely invert the INCO containing vial; 2) inject the saline upwards through the rubber cap; and 3) slowly invert and rotate the vial several times to ensure complete reconstitution of the lyophilized neurotoxin. This procedure is more specific than the current instructions for reconstitution of Xeomin as published in the US Full Prescribing Information, which calls for the saline to be injected in the vial and gently mixed by rotating the vial. Inverting slowly several times, rather than simply rotating horizontally, increases the likelihood that all the powder will be dissolved. It also significantly differs from the reconstitution process for ONA, a process to which many physicians have become accustomed.

Phase I-IV studies, along with retrospective chart review, have shown comparable efficacy and safety between INCO and ONA at a dose conversion ratio of 1:1 in over 2000 patients. The results presented here may provide some insight as to why a small set of studies has reported differences in animals, in one clinical study performed at a non-1:1 dose ratio and a retrospective chart review. In our study, it is quite clear that inadequate reconstitution practices when comparing INCO to ONA led to the differences in clinical efficacy. In our clinical practice, results with INCO using ONA reconstitution practices had led to less than satisfactory results with INCO before a fortuitous change in INCO reconstitution inadvertently occurred. Since the change in reconstitution technique, our favorable experience with over 600 vials of INCO to date strongly suggests that the comparable efficacy between ONA and INCO observed in clinical trials is repeatable in the clinical setting, provided that proper reconstitution is executed.

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DISCLOSURES
Dr. Carey has received compensation for his presentations about Xeomin/Bocouture for Merz Aesthetics (Greensboro, NC).

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