STUDY

Pain Associated With Injection of Botulinum A Exotoxin Reconstituted Using Isotonic Sodium Chloride With and Without Preservative

A Double-blind, Randomized Controlled Trial

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Context: Botulinum A exotoxin is used for various indications, including the treatment of dynamic forehead lines.

Objective: To determine whether injection with botulinum A exotoxin reconstituted with preservative-containing normal saline (isotonic sodium chloride) is less painful than injection with exotoxin that has been reconstituted with preservative-free saline.

Design: Two arms: (1) retrospective study; (2) double-blind, randomized controlled trial.

Setting: A multiple-physician dermatology practice.

Patients: (1) Retrospective study—20 consecutive adult patients presenting for treatment of upper-face dynamic lines; (2) prospective study—15 consecutive adult patients presenting for treatment of upper-face dynamic lines.

Intervention: In prospective study only, one side (left or right) of the face was treated with exotoxin reconstituted with preservative-containing saline, and the other side, with exotoxin reconstituted with preservative-free saline.

Main Outcome Measures: (1) Retrospective study—discomfort at current treatment (with preservative-containing saline) compared with discomfort with most recent prior treatment (with preservative-free saline); (2) prospective study—discomfort on the side treated with preservative-containing saline compared with discomfort on the side treated with preservative-free saline.

Results: (1) Retrospective study—18 (90%) of 20 patients reported that treatment with exotoxin reconstituted with preserved saline was less painful than prior treatment with exotoxin reconstituted with preservative-free saline; (2) prospective study—15 (100%) of 15 patients reported less pain in the side of their face treated with exotoxin reconstituted with preservative-containing saline ($P<.001$). Pain on the preservative-containing side was 54% less. No difference in treatment efficacy between the sides was observed by investigators or patients.

Conclusion: Use of preservative-containing saline to reconstitute botulinum A exotoxin can significantly decrease patient discomfort on injection.

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CONTRARY TO earlier practice beliefs, recent experience indicates that the stability of botulinum A exotoxin is not impaired by reconstitution using preservative-containing isotonic sodium chloride (saline).[1-3] Additionally, the ability to store a vial of botulinum toxin over a period of weeks rather than being forced to use the entire quantity in a single day minimizes waste and has consequent economic advantages. Anecdotal reports from patients and a few physicians have suggested that injections with botulinum toxin reconstituted with preserved saline may also be less painful for patients. The purpose of this study was to compare the pain associated with injections of botulinum A exotoxin reconstituted using saline with and without preservative.

RESULTS

In the RA arm of the study, 20 patients (18 women, 2 men) with a mean age of 49 years (range, 30-61 years) were enrolled. Of these, 18 (90%) noted that the botulinum toxin

PATIENTS, MATERIALS, AND METHODS

The study had 2 arms, a retrospective analysis (RA) and a double-blind, randomized controlled trial (RCT). For the RA, 20 consecutive adult patients presenting to a multiphysician suburban dermatology practice for botulinum A exotoxin treatment of upper-face dynamic lines were invited to participate. For the RCT, 15 consecutive adult patients presenting to the same practice for the same treatment were invited to participate. The RCT patients were told that it had become possible to deliver injections in a manner that some found less painful. It was explained that with their permission, 2 different types of saline would be used, one on each side of the face. If they noted a difference in discomfort, they would be offered whatever formulation they found more comfortable at subsequent visits.

Informed consent was obtained from all patients. None of the consecutively recruited subjects declined to participate. All the enrolled subjects were in good general health.

MATERIALS

Our preservative-free saline was a 0.9% sodium chloride injection (American Regent Laboratories Inc, Shirley, NY). Each milliliter contained 9 mg of sodium chloride and sufficient water for injection. The pH range was 4.5 to 7.0, adjusted with hydrochloric acid and/or sodium hydroxide when necessary. The solution was supplied in 10-mL, single-dose vials and was pregnancy category C.

Our preservative-containing saline was a bacteriostatic 0.9% sodium chloride injection (Abbott Laboratories, North Chicago, Ill). Each milliliter contained 9 mg of sodium chloride and 9 mg of benzyl alcohol added as a bacteriostatic preservative. The pH range was 4.5 to 7.0. The saline might also have contained hydrochloric acid for pH adjustment and was pregnancy category C.

Reconstitution of all vials of botulinum A exotoxin (Allergan Inc, Irvine, Calif) with preservative-free and preservative-containing saline, respectively, was performed by the same investigator (M.A.). There were no differences in technique for the 2 types of saline. For dilution of each vial of botulinum toxin, a fresh vial of diluent with intact flip-off cover was used. The final dilution was 100 U of botulinum toxin per 5 mL of saline. Injections were delivered from 1-mL tuberculin syringes with ½-inch 30-gauge needles (syringe, SlipTip; needle, PrecisionGlide No. 305106; both supplied by Becton Dickinson and Company, Franklin Lakes, NJ).

EXPERIMENTAL DESIGN

The RA was performed to determine whether patients previously injected with preservative-free toxin found the preservative-containing type less painful on subsequent injection. Patients were asked to estimate, on a percentage scale, how much more or less painful the preservative-containing injections were than those from previous treatments without preservative (ie, X percent more painful than pain associated with preservative-free, or Y percent less painful than pain associated with preservative-free).

The RCT was then initiated (**Table 1** and **Table 2**), with patients randomly assigned to receive toxin reconstituted with preserved saline to one side of the face (left or right) and toxin reconstituted with preservative-free saline to the other side. Injections were for dynamic lines of the upper face. Patients received treatment for glabellar, forehead, and/or lateral orbital creases. Injection of one side of the face was completed before injections on the other side were begun. For every second patient, the first set of injections was with preservative-free botulinum toxin. Immediately after treatment completion, patients were asked if injections on both sides had been equally uncomfortable, or if one side had been more or less painful than the other. If one side had been more painful, they were asked to estimate the pain on the preservative-containing side as a percentage of that on the preservative-free side (eg, assuming the right side was preservative-free: "How much more or less painful, in percentage terms, were the injections on the left side than those on the right side?").

BLINDING TECHNIQUE

For the RCT, 1 investigator (M.A.) prepared preservative-containing and preservative-free botulinum toxin in a sequestered space. For each patient, both types were drawn up in identical syringes with identical needles and hubs. There were no distinguishing marks. All injections were performed by another investigator (J.S.D.), who was serially handed syringes by the preparer (M.A.) and instructed on which side to begin injecting. Record keeping by the preparer was done several feet behind the back of the injector. After treatment, the injector asked the patient to estimate relative discomfort; this eliminated the risk that a query by the preparer, who knew which side was treated with preservative-containing solution, could bias the response.

injections that they had just received (reconstituted with preserved saline) had been less painful than their most recent previous treatments (reconstituted with preservative-free saline). Except for 1 patient treated for axillary hyperhidrosis, all of the patients' current and prior treatments were for dynamic creases of the upper face. The patients who noticed decreased pain with the current treatments estimated, on average, that these treatments were 55% (range, 20%-80%) less painful than those in the past. The 2 remaining patients, to the best of their recollections, recalled no significant difference in pain between the successive treatments.

In the RCT arm of the study, 15 women with mean age of 48 years (range, 33-64 years) were enrolled. Of these, 15 (100%) reported that they experienced less pain in the side of the face treated with preservative-containing botulinum toxin injections than in the side treated with preservative-free toxin. Statistical analysis was performed by application of the binomial probability distribution. The results were determined to be highly statistically significant ($P<.001$) despite the small sample size.

The average pain level on the preservative-containing side was 54% less severe than on the preservative-free side

Table 1. Patient Assessment of Discomfort Associated With Current Preservative-Containing Botulinum Toxin Injection Treatment Compared With Immediately Previous Preservative-Free Treatment*

Patient No./ Sex/Age, y	Areas Treated	Current Treatment More/Less Painful	% Change Compared With Previous Treatment
1/F/59	Glabella	Same	0
2/F/36	Glabella, forehead, perioral	Less	50
3/F/52	Eyes	Less	25
4/F/58	Glabella, forehead, eyes	Less	75
5/F/51	Glabella, forehead, eyes	Less	40
6/F/40	Glabella, forehead, eyes	Less	80
7/F/58	Glabella, forehead, eyes	Less	50
8/M/30	Axilla	Less	80
9/F/51	Glabella, forehead, eyes	Less	60
10/F/61	Glabella, forehead	Less	50
11/F/43	Glabella	Same	0
12/F/44	Glabella, forehead, eyes	Less	60
13/M/51	Glabella	Less	70
14/F/39	Forehead, eyes	Less	50
15/F/57	Glabella, forehead, eyes	Less	30
16/F/51	Glabella, forehead, eyes	Less	75
17/F/47	Glabella, forehead	Less	40
18/F/60	Eyes	Less	50
19/F/55	Glabella, forehead	Less	60
20/F/38	Glabella	Less	50

*Previous treatments were 3 to 6 months before current treatment.

(range, 33%-80%). Subjective pain assessments of the preservative-free solution communicated a greater degree of "piercing," "stinging," "sharpness," and "pinching," whereas the preservative-containing side "barely hurt" or "didn't hurt at all."

COMMENT

The manufacturer of botulinum A exotoxin (Allergan Inc) has historically encouraged reconstitution of the desiccated form with unpreserved saline. This formulation was experimentally shown to be efficacious and stable in initial clinical trials. While alternative types of saline have not been reported to be any less effective, Allergan officially continues to recommend dilution with preservative-free saline. Technical support personnel at Allergan also assert that use of preservative-free saline may result in less painful injections (personal telephone communication, Botox physician help line, Allergan Inc, May 13, 2001).[4] Roger Aoki, MD, a researcher at Allergan, has found that botulinum A exotoxin reconstituted with preserved saline is stable with refrigeration for 5 weeks (personal communication, 2000). Others have also found that dilution with preserved saline seems to leave intact the efficacy of botulinum toxin.[5] While few physicians store reconstituted toxin for more than a few days, the ability to do so for limited intervals conserves material, reduces physician and patient costs, and permits more convenient scheduling for patients. Refrigeration of botulinum toxin reconstituted with preserved saline has enabled use of a given vial over a period of several days to weeks.

Table 2. Patient Assessment of Discomfort Associated With Right/Left Split-Face Botulinum Toxin Injection Treatment With Preservative-Containing and Preservative-Free Solution

Patient No./ Sex/Age, y	Areas Treated	Preservative-Containing Side/ More or Less Painful	% Change Compared With Preservative-Free
1/F/54	Glabella, forehead, occiput	Left/less	33-50
2/F/52	Glabella, eyes	Left/less	50
3/F/40	Glabella, eyes	Right/less	40
4/F/47	Glabella, forehead, eyes, temples	Left/less	50
5/F/44	Glabella, forehead, eyes	Left/less	60
6/F/48	Glabella, eyes	Right/less	60
7/F/49	Glabella, forehead, eyes	Right/less	80
8/F/51	Glabella, forehead, eyes	Left/less	50
9/F/64	Glabella	Left/less	50
10/F/57	Glabella	Right/less	40-50
11/F/33	Eyes	Left/less	75
12/F/41	Glabella, forehead, eyes	Right/less	60
13/F/50	Glabella, forehead	Left/less	50
14/F/40	Glabella, eyes	Right/less	40
15/F/57	Forehead, eyes	Right/less	50-60

This study demonstrates that patient comfort is also enhanced by botulinum toxin hydrated with preserved saline (Tables 1 and 2). Both the RA and the RCT showed a statistically significant lower level of injection pain associated with the use of preserved saline. More importantly, the difference was clinically significant. Patients noticed a dramatic reduction in unpleasant sensation, which they quantified as an approximately 50% diminution. The estimates of pain reduction were strikingly similar for both the RA and RCT comparisons. No patient preferred the botulinum toxin diluted with preservative-free saline.

Interestingly, most patients described the initial punctures with the preservative-free syringes as feeling sharper, as if with a different needle, even though exactly the same hardware was used to deliver both types of injections. Patients also reported increased pain and pressure associated with preservative-free toxin once the needle was in and the plunger was depressed. The 1 patient who received treatment for axillary hyperhidrosis encountered the same sensations. The results do not seem unique to injection of the upper face.

Follow-up self-report indicated that none of the treated patients in either arm of the study noticed any difference in efficacy between the preservative-containing and preservative-free injections. Similarly, in all instances in which left-right treated patients were seen in the clinic for other reasons within 4 months of the injection protocol, the treating physicians observed no differences between the 2 sides either at rest or with voluntary contraction.

Investigations were performed to confirm that the pain-muting effects of bacteriostatic saline observed in this study were not due to differences in pH between the bacteriostatic and preservative-free saline. Manufacturers of both the diluents used were contacted and queried as to the typical pH of their products. The pH

range was reported to be 4.5 to 7.0 for the preservative-containing as well as the preservative-free saline made by each company. Additionally, Abbott Laboratories noted that their bacteriostatic saline had a usual tested pH of 5.0, and their preservative-free saline, 5.6 (personal communication, Abbott Laboratories, Pharmacy, Hospital Division, May 14, 2001). Computations by American Regent Laboratories revealed a mean tested pH of 5.33 for all batches of 10-mL preservative-free saline vials produced at their facility from 1999 through May 2001 (personal communication, American Regent Laboratories technical support, May 14, 2001). Apparently, preservative-free and preservative-containing saline do not differ significantly in terms of pH. Slightly greater acidity is the norm for preservative-containing saline, and while relative acidity suggests more pain on injection, such an outcome was not observed.

The remaining major difference between the 2 categories of saline we studied was the presence or absence of benzyl alcohol. There is substantial evidence that benzyl alcohol has anesthetic properties. At least 4 double-blind, randomized controlled trials have been conducted to compare in the same individuals the pain experienced on subcutaneous injection of otherwise identical solutions using preservative-free normal saline and benzyl alcohol–containing normal saline.[6-9] In one such study,[7] preservative-free and preservative-containing saline were injected without combination with other agents to facilitate anesthesia before intravenous catheter placement. Five other substances, including 1% lidocaine, 1% lidocaine with preservative, and alkalinized 1% lidocaine with preservative, were also examined in similar fashion. Of the 7 injectants, benzyl alcohol in normal saline was associated with the lowest mean±SD pain scores (0.61±0.11, on a 10-cm visual analog pain scale), and normal saline, with the highest scores (3.97±0.18). The difference was statistically significant.

In a similar study,[9] 20 healthy volunteers were injected with each of 6 solutions, among which were normal saline, preserved normal saline, 0.2% lidocaine in normal saline without preservative, and 0.2% lidocaine in preserved saline. Degree of anesthesia was assessed by pinprick every minute for 20 minutes. Normal saline alone caused the most pain, and both preservative-containing saline and lidocaine with preservative-containing saline caused the least pain. Adequate anesthesia was obtained for 4 minutes with preservative-containing saline alone. In the dermatology literature, Williams and Howe[8] graded the pain induced by intradermal and subcutaneous injections of 1% lidocaine diluted with preservative-free and benzyl alcohol–containing saline. On average, the 20 subjects found the preservative-containing lidocaine 27% less painful on injection. Duration of anesthesia was 29% longer with the preservative-containing solution.

The anesthetic action of benzyl alcohol has also been investigated by physicians trying to minimize the pain of subcutaneous injection with recombinant human erythropoietin (epoetin alfa) in patients with renal disease.[10] Twenty-eight hemodialysis patients received epoetin injections diluted with saline with and without benzyl alcohol. Results showed a statistically significant difference in pain perception at times 0, 10, and 15 minutes on both a visual analog scale and a verbal descriptive pain scale. Preservative-containing solutions were less painful in all instances. Patient differences were also noted, with several patients reporting no pain with the unpreserved saline.

Numerous other studies with less symmetric design have corroborated the anesthetic properties of benzyl alcohol.[10-16] For pain relief during intravenous line placement, intradermal preserved saline alone has been shown to be as effective as intradermal 1% lidocaine hydrochloride.[10] Intradermal injections of preservative-containing saline with 1:100 000 epinephrine have been found to be 48% less painful than injections of unpreserved saline alone ($P=.008$).[11] Cutaneous anesthesia in patients allergic to lidocaine may be better achieved with injection of preserved saline with epinephrine than with injection of 1% diphenhydramine.[12] The former was statistically significantly less painful on injection (5 mm vs 55 mm median pain score on 100-mm visual analog pain scale) and provided equally long-lasting anesthesia.

For eyelid surgery, 2% lidocaine with 1:100 000 epinephrine diluted 1:9 with 0.9% bacteriostatic saline caused less discomfort on injection than both plain 2% lidocaine with 1:100 000 epinephrine and 2% lidocaine with 1:100 000 epinephrine buffered 1:9 with 8.4% sodium bicarbonate.[15] According to patient reports, the level of anesthesia induced following injection did not differ for the various solutions. Benzyl alcohol–containing saline also seems to be a useful anesthetic in children. In a randomized convenience sample of 99 children older than 6.8 years seen in an emergency room, saline with benzyl alcohol and 1% lidocaine were equally effective as intradermal anesthetics for intravenous line placement.[16]

Indeed, normal saline with benzyl alcohol has all the qualities of an ideal anesthetic agent.[17] Pain on application and pain on venipuncture are both low, as is cost, and the substance is convenient to use. Lidocaine may provide longer and better anesthesia, but its pain on application is greater.

Benzyl alcohol–containing saline should not be injected intrathecally[18,19] and has been associated with toxic effects in newborns.[20] Obviously, neither of these caveats would preclude its use in reconstituting botulinum toxin for intramuscular or intradermal injection in adults.

In summary, this study indicates that reconstitution of botulinum toxin with preservative-containing saline can markedly decrease patient discomfort at the time of injection. The difference is statistically and clinically significant. Given the increasing evidence, confirmed in our investigation, that preservative-containing and preservative-free botulinum toxin preparations are equally safe and effective, physicians who do not do so at present should consider using the preservative-containing solution. Indeed, since the completion of this study, we have discontinued use of preservative-free saline to dilute botulinum toxin in our practice and have noticed a sustained increase in patient satisfaction associated with the change. Minimizing patient pain is an impor-

tant physician responsibility. Elective procedures such as injections for dynamic creases should be particularly devoid of unnecessary suffering.

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