

Facial Rejuvenation After Intradermal Botulinum Toxin: Is it Really the Botulinum Toxin or is it the Pricks?

RINKY KAPOOR, MD,* DEBRAJ SHOME, MD, FRCS (GLASGOW), MBA,[†] VANDANA JAIN, MD,[‡] AND RAJESH DIKSHIT, MD[§]

BACKGROUND The use of intradermal botulinum toxin A (onabotulinumtoxinA) remains a relatively new technique and is an off-label cosmetic application for facial skin rejuvenation. There is little documented clinical evidence of the objective benefits of this therapy.

PURPOSE To determine whether intradermal facial onabotulinumtoxinA injection has any benefits.

STUDY DESIGN Interventional, comparative, split face clinical trial.

METHODS Informed consent was obtained from 10 physicians. One half of the physician's faces were randomly injected with onabotulinumtoxinA (2 U/0.1 mL; 30 facial injections on half of the face, each 0.1 mL) intradermally and the other half of the face with normal saline (30 facial injections on half of the face, each 0.1 mL). The injecting clinician and the subjects were blinded to the contents of the syringes. One and 4 weeks later, two neutral, blinded observers assessed the subjects in person. The patients were also photographed in ambient light surroundings and the same observers compared the halves of their faces in photographs and rated them on a scale of -4 to +4.

RESULTS Global improvement in skin texture and tightness was noted in the post-treatment photographs (the skin appeared to be tenser and smoother), although there was no difference between the two groups and, hence, the changes could not be clinically ascribed to the intradermal botulinum toxin injections. No other meaningful clinical difference could be demonstrated between the two sides of the face, in any of the 10 subjects, in person or in photographs. The small study sample precluded formal statistical analysis.

CONCLUSION Intradermal botulinum toxin A injection does not appear to have any benefit in facial rejuvenation.

The authors have indicated no significant interest with commercial supporters.

Botulinum toxin A (onabotulinumtoxinA) is a powerful neurotoxin first introduced by Dr. Alan Scott for treating eye muscle disorders in the late 1980s; since then, this toxin has been widely used for muscle spasm disorders and other indications. With the satisfactory results of several research studies published, the Food and Drug Administration has approved onabotulinumtoxinA to treat glabellar lines, blepharospasm, strabismus, hemifacial spasms, cervical dystonia, and spasticity. Now, injection with onabotulinumtoxinA has become the most popular cosmetic procedure, and additional aesthetic applications have been discovered in recent years.¹

Currently, many off-label cosmetic applications of onabotulinumtoxinA are under evaluation. Based on its mechanism in blocking the release of several neurotransmitters, especially acetylcholine, onabotulinumtoxinA has been used in fields such as pain control and hyperhidrosis treatment.¹ The use of intradermal botulinum toxin remains a relatively new technique.² Some case reports have highlighted the effects of intradermal onabotulinumtoxinA in decreasing the size of skin pores, decreasing sebum production, improving the texture of skin, and causing "mini facelift-like" effects.² The purported mechanism of these actions is local blockade of

*Departments of Cosmetic Dermatology and [†]Facial Plastic Surgery, Apollo Hospitals, Hyderabad, India; [‡]Department of Cornea & External Diseases, Aditya Jyot Eye Hospital Pvt Ltd, Mumbai, India; [§]Department of Biostatistics, Tata Memorial Hospital, Mumbai, India.

acetylcholine receptors by onabotulinumtoxinA because acetylcholine plays a significant role in the regulatory mechanism of skin processes.² It has been claimed that increased collagen synthesis causes the effect.¹ An article² reported preliminary data suggesting that intradermal onabotulinumtoxinA may play a role in decreasing sebum production, although the author agreed that the trial could not determine whether onabotulinumtoxinA shrinks the pores.² By and large, the documentary evidence of the use and the benefits of intradermal onabotulinumtoxinA in the face is limited.

What makes this debate even more interesting is that percutaneous needle pricks themselves have been reported to create multiple microbruises in the dermis, and these actually initiate the complex cascade of growth factors that eventually result in collagen production.³ Orentreich and colleagues³ independently described subcision or dermal needling by pricking the skin with a needle to scarify the dermis and build up connective tissue under scars and wrinkles. The modality of action of this technique is that the needle pricks break old collagen structures and the trauma induces an inflammatory cascade, scar collagen is broken down, and new collagen is replaced once again under the epidermis.³

The lack of substantial, well-documented evidence led us to conceive a comparative trial in which halves of 10 physicians' faces were injected with multiple intradermal jabs of onabotulinumtoxinA (2 U/0.1 mL) and the other halves of the faces were injected with multiple intradermal injections of normal saline (0.1 mL). The objective of our study was to determine whether intradermal botulinum toxin injections have any adjuvant benefits over injections of normal saline, which served as a control (percutaneous pricks).

Methods

Each vial of onabotulinumtoxinA containing 100 U of *Clostridium botulinum* toxin type A with human serum albumin and sodium chloride (Botulinum

toxin A, purified neurotoxin complex, Allergan, Irvine, CA) was reconstituted with 5 mL of sterile, preservative-free saline to achieve a concentration of 2 U/0.1 mL. Ten healthy Indian physician volunteers (5 men, 5 women) aged 24 to 36 (Table 1) were enrolled in this double-blind, placebo-controlled, split-face study after signing an informed consent for this off-label use of onabotulinumtoxinA. We excluded subjects who had a history of onabotulinumtoxinA injections or cosmetic procedures performed within the past 12 months. Each patient had a pretreatment photograph taken in ambient light surroundings. Two dermatologists evaluated the skin texture, pore size, skin tightness, and sebum production of each volunteer. Sebum production was measured using a sebumeter at three sites: 1 inch over each eyebrow, at the center of the nasolabial fold, and 0.5 inches below the corner of the mouth. Sebum was measured in μg of sebum/cm² of skin.

Sides of the face of each patient were randomly assigned for injection, postrandom allocation,

TABLE 1. Summary Statistics for Variables Under Study

<i>Variable</i>	<i>Mean $\pm$ Standard Deviation</i>
Age	31.8 $\pm$ 3.1
Observed clinically	
Skin texture	
Botulinum toxin side	1.6 $\pm$ 0.8
Saline-only control side	1.6 $\pm$ 0.9
Skin tightness	
Botulinum toxin side	1.4 $\pm$ 0.7
Saline-only control side	1.5 $\pm$ 0.7
Pore size	
Botulinum toxin side	0.6 $\pm$ 0.6
Saline-only control side	0.4 $\pm$ 0.5
Sebum production	
Botulinum toxin side	0.8 $\pm$ 0.4
Saline-only control side	0.7 $\pm$ 0.5
Photographic documentation	
Skin tightness	
Botulinum toxin side	0.7 $\pm$ 0.7
Saline-only control side	0.9 $\pm$ 0.7
Pore size	
Botulinum toxin side	0.4 $\pm$ 0.5
Saline-only control side	0.5 $\pm$ 0.5

and allocation concealment: one side to onabotulinumtoxinA intradermal injections and the other side to normal saline intradermal injections as a control. The injecting physician and the patients were blinded to this choice, as well as to the contents of the injecting syringes. Topical anesthesia in the form of a eutectic mixture of local anesthetic cream (AstraZeneca, Karlskoga, Sweden) was applied 1 hour before the injections. A 30-gauge needle was used to inject the materials intradermally. The treatment areas were staggered at 1-cm intervals to cover the entire half of the face. Similar areas were injected on both sides of the face. The injection volume was 0.1 mL per treatment site. The end point of the injection was a subepidermal wheal-like swelling. The total dose of onabotulinumtoxinA injected per person was 30 U. The patients were reviewed 1 and 4 weeks after the injections.

Two neutral, blinded dermatologists assessed the patients in person and scored each half of the face on a scale of -4 to $+4$ compared with the pretreatment baseline score reading of 0. (Both observers mentioned details individually for each side of the face to serve as a bench mark after treatment to assess the improvement or the worsening on the score ranging from -4 to $+4$.) The patients were also photographed in ambient light surroundings and two dermatologists compared the halves of the faces in photographs using Photoshop 8.0 (Adobe Systems Incorporated, San Jose, CA) on a scale of -4 to $+4$ once again. The photographs were also compared with the pretreatment photographs and rated. Skin texture, pore size, and skin tightness were evaluated in the photographs 1 and 4 weeks after the procedure. The physicians evaluated skin texture and tightness on a scale of -4 to $+4$ ($4 = 76\text{--}100\%$ improvement, $3 = 51\text{--}75\%$ improvement, $2 = 26\text{--}50\%$ improvement, and $1 = 1\text{--}25\%$ improvement). Deterioration in the above parameters was similarly evaluated on the negative scale. Changes in sebum production was measured using a sebumeter and rated similarly. Measurements with the instrument were taken at the same spot before and 1 and 4 weeks after the injection of onabotulinumtoxinA.

The sebum measurement was displayed as a number. Then, based on the numerical value displayed in the sebumeter before and after the injection of onabotulinumtoxinA or saline, we assessed the percentage of improvement or worsening of the sebum production from baseline. This percentage of improvement or worsening was then graded in a similar manner on a scale of -4 to $+4$. Change in pore size was also evaluated on clinical and photographic examination according to the same 8-point scale. The two evaluating dermatologists who were blinded to the assignment also comparatively scored the effect on skin tightness and texture, pore size, and sebum production from -4 to $+4$ for both sides of the face.

Results

None of the enrolled subjects experienced significant adverse effects, such as allergic reaction, facial palsy, or severe paralysis of muscles adjacent to the point of injection during or after this study. A mild to moderate stinging sensation was noted. In all subjects, the pain was tolerable and was comparable between both sides of the face.

Objectively, there was no difference clinically between the skin on both sides of the face 1 or 4 weeks after the injections (Tables 2–5), although global improvement in skin texture and tightness was noted between the pre- and post-treatment photographs; the skin appeared to be tenser and smoother (Figures 1 and 2), although no significant difference could be demonstrated in the above response between the onabotulinumtoxinA-treated side and the saline-injected side. The sample size of the study was small, and this could be one reason for the failure to demonstrate statistical significance. The results were obtained on the basis of the average of the grades of improvement scores of the observers and photographic assessment. Specimens of the facial skin of the subjects could not be obtained for histological examination because of lack of patient consent.

TABLE 2. Grades of Clinical Improvement After 1 Week (Mean of Values of Two Observers)

Age	Sex	Skin Texture		Skin Tightness		Pore Size		Sebum Production	
		Botulinum Toxin	Saline-only Control	Botulinum Toxin	Saline-only Control	Botulinum Toxin	Saline-only Control	Botulinum Toxin	Saline-only Control
31	F	2	2.5	2.5	2	1	1	1	1
29	M	1	1	1	1	0	0	0	1
29	F	2	1	1	1	1.5	1	1	1
32	M	2.5	2	2	2.5	1	1	1	1
36	M	2	1	2	1	1	0	1	0
27	M	0	0	0	0	0	0	1	0
30	F	1	1.5	1.5	2	0	0	1	1
35	F	2	2	1	1.5	0	0	1	1
36	F	2.5	3	2	2	1	1	1	1
33	M	1	2	1.5	2	0	0	0	0

Discussion

The use of onabotulinumtoxinA continues to revolutionize the medical field with new applications. OnabotulinumtoxinA injections represent a targeted therapy with minimal systemic effects.² Over the years, onabotulinumtoxinA has been successfully used for a wide variety of indications such as facial hyperkinesis (blepharospasm, hemifacial spasm), complex dystonias (oromandibular dystonia, spasmodic dystonia, cervical dystonia), gustatory sweating, hypersalivation, crocodile tears,⁴ and hyperhidrosis,⁵ but onabotulinumtoxinA is used most widely for its application in cosmetic correction, namely in the reduction in intensity of frown

and wrinkle lines of the forehead, glabella, and lateral periorbital area. It has also been used to reduce the intensity of chin and upper lip wrinkling, nasolabial folds, and platysma neck bands.⁶ Patient satisfaction after facial recontouring with onabotulinumtoxinA treatment is consistently high.⁷

We performed a pilot trial to assess the utility of intradermal onabotulinumtoxinA in facial skin rejuvenation. In this pilot study, no significantly different skin rejuvenating effect of the intradermal onabotulinumtoxinA injection from saline injections could be demonstrated. According to objective assessment by two dermatologists, there was no

TABLE 3. Grades of Photographic Assessment Improvement After 1 Week (Mean of Values of Both Observers)

Age	Sex	Skin Tightness		Pore Size	
		Botulinum Toxin	Saline-only Control	Botulinum Toxin	Saline-only Control
31	F	1	1.5	1	1
29	M	0	0.5	0	0.5
29	F	0	0	0	1
32	M	1	1	1	1
36	M	1	0	0	0
27	M	0	0.5	0	0
30	F	1	1	0	0
35	F	1	1	0	0
36	F	2	2.5	1	1
33	M	0	1	0.5	0

TABLE 4. Grades of Clinical Improvement After 4 Weeks (Mean of Values of Two Observers)

Age	Sex	Skin Texture		Skin Tightness		Pore Size		Sebum Production	
		Botulinum Toxin	Saline-only Control	Botulinum Toxin	Saline-only Control	Botulinum Toxin	Saline-only Control	Botulinum Toxin	Saline-only Control
31	F	2.5	2	2.5	2	1	1	0	0
29	M	0	1.5	1.5	1.5	0	0	0	1
29	F	2.5	1.5	1.5	1	1.5	1	1	0
32	M	2	2.5	2.5	2	1	1	0	1
36	M	2	1	2.5	1.5	1	0	1	0
27	M	1	1	0	0	1	1	0	0
30	F	1	1	1	2.5	1	0	1	0
35	F	2.5	2.5	1.5	2.5	1	0	0	1
36	F	2	2.5	1.5	2.5	1	1.5	1	0
33	M	1	2	1	2	0	1	0	0

significant difference between the conditions of treated skin on both sides of the face. The post-therapy photographs seemed to suggest an improvement in the skin texture and the skin tightness, but this was so on both halves of the face. No difference could be demonstrated between the two sides of the face after therapy, suggesting that the improvement was not dependent on the drug injected. Reports of smoothening of wrinkles, as is claimed in certain studies of intra-dermal onabotulinumtoxinA,¹ can be considered to be due to intramuscular diffusion of the intradermally injected onabotulinumtoxinA and is probably not best

evaluated in a study evaluating intradermal onabotulinumtoxinA. Nevertheless, we did not see any wrinkle-reducing effect due to onabotulinumtoxinA per se, probably indicating that we were in the right plane (i.e., intradermal), that the intradermal onabotulinumtoxinA cleaved the dermal lamellae and did not have significant intramuscular diffusion in our patients. The findings from our study suggest that the improvement in skin texture might be due to contribution of collagen neosynthesis, although immunochemistry to demonstrate this could not be performed in this study. A reasonable explanation could be the direct induction of neocollagenesis by

TABLE 5. Grades of Photographic Assessment Improvement After 4 Weeks (Mean of Values of Both Observers)

Age	Sex	Skin Tightness		Pore Size	
		Botulinum Toxin	Saline-only Control	Botulinum Toxin	Saline-only Control
31	F	1	1	1.5	1.5
29	M	0	0	0	0
29	F	0	0	0	0
32	M	0	0	0	0
36	M	1	0	0.5	0.5
27	M	0	0.5	0.5	0.5
30	F	1	0	0.5	0.5
35	F	0	0	0.5	1
36	F	1.5	1.5	1	1
33	M	0.5	1	0.5	1

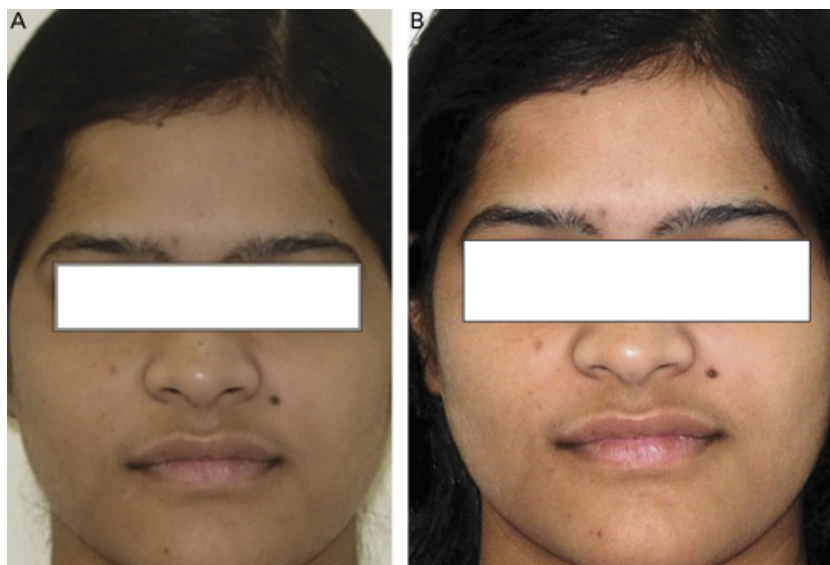


Figure 1. (A) Preprocedure photograph of a female patient. (B) Photograph 4 weeks after the procedure. The right side of the face was injected with intradermal saline and the left side with intradermal botulinum toxin A.

minimal trauma through the process of injection. The mechanical stretching can stimulate dermal fibroblasts to secrete new collagen, which was also demonstrated in intradermal injection with hyaluronic acid.¹ The inclusion of a negative control (no treatment) cohort in the study design may have strengthened this trial, although it was extremely

difficult to get more volunteers for another treatment cohort in a trial of this nature. We also tried to fit a logistic regression model using drug (onabotulinumtoxinA vs no onabotulinumtoxinA) as a dependent variable and skin texture, skin tightness, and sebum as independent variables, but because of the small number of observations (10), the interval



Figure 2. (A) Preprocedure photograph of a female patient. (B) Photograph 4 weeks after the procedure. The left side of the face was injected with intradermal saline and the right side with intradermal botulinum toxin A.

estimates were too wide to be of any use for interpretation, and the data could not be tabulated.

Our results support the clinical trial conducted by Amin and colleagues,⁸ who performed a mesotherapy trial on 10 subjects. Ten subjects were subjected to four sessions of mesotherapy involving multiple injections of a multivitamin and hyaluronic acid solution. Treatment was conducted at four monthly intervals. All subjects had pre- and post-treatment photographs and skin biopsies, and the skin biopsies were evaluated with routine histology, mucin and elastin stains, and electron microscopy. Patient surveys were also evaluated. In this trial, evaluation of photographs at 0, 3, and 6 months revealed no significant clinical differences. Light microscopic examination of pre- and post-treatment specimens showed no significant changes, and electron microscopic analysis of collagen fibers showed slightly smaller collagen fibrils, which had decreased from a mean of 59 to 48 nm. The authors concluded that multivitamin and hyaluronic acid solution facial mesotherapy does not appear to provide any significant benefit. Because smaller-diameter collagen fibers are frequently associated with synthesis of new collagen and the presence of procollagens or type III collagen and because newly synthesized collagen follows diverse stimuli, including inflammatory or thermal injury, and frequently is associated with the presence of a repair zone, the authors further concluded that it is most likely that the most basic form of mesotherapy results in no significant clinical, histologic, or ultrastructural changes in collagen fibers and that a majority of the effects after mesotherapy may be merely an inflammatory reaction to the pricks.⁸

In conclusion, based on our limited number of cases, the skin rejuvenating effect of intradermal onabotulinumtoxinA, for which it is being used as a non-Food and Drug Administration–approved indication, was not conclusive in this study. The effects of intradermal onabotulinumtoxinA have yet to be studied in detail. The limitation of this trial is that it was limited to photographic analysis and clinician

observations, and it is probable that more sophisticated digital skin analysis and profilometry, and perhaps even indirectly straightforward patient-reported outcomes and feedback, might have captured the less perceptible effects of botulinum toxin on skin surface anatomy and physiology. As reported by Kurzen and Schallreuter,⁹ the acetylcholine receptor is not only present on neurons, but also can be found on the surface of melanocytes, keratinocytes, and other dermal tissue. It is reasonable to suspect that a versatile effect may be produced on adjacent tissue components after onabotulinumtoxinA injection.¹ We need additional placebo-controlled studies, with more cases, longer follow-up periods, more precise quantitative methods, and perhaps higher dosages, before we can recommend intradermal onabotulinumtoxinA or meso-onabotulinumtoxinA, as it is called, to the patient.

Conclusion

Intradermal botulinum toxin injection does not appear to have any benefit for facial rejuvenation. The purported benefits of skin rejuvenation are probably due to the collagenesis caused by the needle pricks.

References

1. Chang SP, Tsai HH, Chen WY, et al. The wrinkles soothing effect on the middle and lower face by intradermal injection of botulinum toxin type A. *Int J Dermatol* 2008;47:1287–90.
2. Shah AR. Use of intradermal Botulinum toxin to reduce sebum production and facial pore size. *J Drugs Dermatol* 2008;7:847–50.
3. Fernandes D, Signorini M. Combating photoaging with percutaneous collagen induction. *Clin Dermatol* 2008;26:192–9.
4. Rohrbach S, Laskawi R. Botulinum toxin in otorhinolaryngology. *HNO* 2004;52:635–41.
5. Bhidayasiri R, Truong DD. Evidence for effectiveness of botulinum toxin for hyperhidrosis. *J Neural Transm* 2008;115:641–5.
6. Beedetto AV. The cosmetic uses of Botulinum toxin type A. *Int J Dermatol* 1999;38:641–55.
7. Fagien S, Carruthers JD. A comprehensive review of patient-reported satisfaction with botulinum toxin type A for aesthetic procedures. *Plast Reconstr Surg* 2008;122:1915–25.

8. Amin SP, Phelps RG, Goldberg DJ. Mesotherapy for facial skin rejuvenation: a clinical, histologic, and electron microscopic evaluation. *Dermatol Surg* 2006;32:1467–72.
9. Kurzen H, Schallreuter KU. Novel aspects in cutaneous biology of acetylcholine synthesis and acetylcholine receptors. *Exp Dermatol* 2004;13:27–3014.

Address correspondence and reprint requests to: Debraj Shome, MD, FRCS, MBA, Department of Facial Plastic Surgery, Apollo Hospitals, Apollo Health City, Hyderabad, India, or e-mail: debraj_shome@yahoo.com