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A Double-Blind, Split-Face, Randomized Study on the Effects and Safety of Intradermal Injection of Botulinum Toxin A (Incobotulinum Toxin A) in the Cheek

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Background: Intradermal injection of botulinum toxin A (BTXA) is used for cosmetic purposes without strong evidence for clinical use, as opposed to intramuscular injection.
Objective: To evaluate the efficacy and safety of intradermal injection of incobotulinum toxin A (iBTXA) in the cheeks.
Methods: We conducted a study with 18 volunteers who received intradermal injection of iBTXA into one cheek and normal saline into the contralateral side as a control. Volunteers visited the clinic at weeks 2, 4, 8, and 12 after injection. At each visit, pores and wrinkles were evaluated by a facial analyzer, sebum secretion by a sebumeter, skin texture by both volunteers and clinicians, and wrinkles of the nasolabial fold were graded with photographic reviews.
Results: There were no significant effects on the wrinkles of the infraorbital area and sebum secretion. However, there were significant improvements in the wrinkles of the nasolabial fold and skin texture on the iBTXA injected side. The effects on the wrinkles of the nasolabial fold lasted 12 weeks, and those on skin texture lasted 8 weeks. Improvement in the pore size was observed only at week 2. No serious adverse events were reported except one volunteer who complained of facial palsy after the injection of 30 units of iBTXA in one cheek. However, injection of 20 units of iBTXA in one cheek was not associated with any adverse events.
Conclusion: Intradermal injection of iBTXA, could provide clinical benefits for skin texture and wrinkles overcoming the skin prick effect without obvious side effects.

Keywords: Botulinum toxins, Cheek, Intradermal injections

INTRODUCTION

Botulinum toxin A (BTXA) is a neurotoxin that inhibits muscle contraction by blocking several neurotransmitters, especially acetylcholine[1]. Based on its mechanism of action, BTXA improves facial wrinkles and was considered as the first line of treatment especially for glabella and periocular wrinkles[2-5]. Intramuscular BTXA injection has been administered not only for contouring of the face and wrinkles, but also for treatment of cervical dystonia, facial palsy, bruxism, and neuropathic pain[6-9]. In addition, it is used as an intradermal injection for treatment of hyperhidrosis on the hand, axilla, and scalp[10]. Both intramuscular and intradermal injections are possible depending on the purpose.

From the late 2000s, intradermal injections began to be applied in cosmetic procedures to improve wrinkles because of the lifting effects on the cheeks and perioral areas[11]. Clinicians claimed that intradermal injection of BTXA has similar efficacy in improving wrinkles compared to muscular injection by not only improving wrinkles, but also smoothening

overall skin texture and reducing sebum secretion and the size of pores[2,3,11-13]. Previous studies demonstrated that these effects are caused by increased collagen synthesis upon histological examination[2,11]. Other studies reported that blockade of cholinergic signaling on local muscarinic receptors within the sebaceous gland and construction of arrector pili muscles through intradermal injection of BTXA affect sebum excretion and pore size[1,2,11]. However, one study showed that the effects are caused by the needle prick and not the intradermal injection of BTXA[12]. A reasonable explanation is that percutaneous needle pricks create multiple areas of microtrauma in the dermis and damage collagen structures to stimulate the complex cascade of growth factors that eventually results in new collagen synthesis. The effect of intradermal injection of BTXA in the skin has yet to be concluded.

To evaluate the efficacy and safety of intradermal injection of BTXA on pores, wrinkles, and skin texture with objective evidence, we conducted a comparative split-face study using incobotulinum toxin type A (iBTXA), which does not contain any complexing proteins unlike onabotulinumtoxin A or abobotulinumtoxin A.

MATERIALS AND METHODS

Study design and subjects

This study is a prospective, double-blind, split-face study. The study was approved by the Institutional Review Board at Samsung Medical Center (2019-07-044) and has been registered in Clinical Research Information Service on April 9, 2020 (http://cris.nih.go.kr, KCT0004952). This study took place at the Samsung Medical Center in Seoul, Republic of Korea. Informed consent was appropriately obtained with the voluntary consent of the volunteers, and we received the patient's consent form about publishing all photographic materials. Volunteers with contraindications for BTXA injection such as preexisting neuromuscular disease (myasthenia gravis, amyotrophic lateral sclerosis, Lambert-Eaton Syndrome, etc.) or medication that could be affected by BTXA injection were excluded. Volunteers with treatment history (filler injection, tissue grafting, radio-frequency treatment, etc.) that could influence the evaluation of the clinical trial were also excluded.

Botox preparation and treatment

Coretox® (incobotulinum toxin [iBTXA], Clostridium botulinum toxin type A, purified neurotoxin complex; Medytox, Seoul, Korea) was injected at 1-cm intervals on one side of the cheek from under the lower eyelid to the corner of the mouth. A single injection volume of 0.025 ml (0.5 U per spot) was injected intradermally, and no more than 20 U of iBTXA in total was administered except in one volunteer who was injected with a total of 30 U of iBTXA on one side of her face. On the contralateral side, the same volume of normal saline was injected intradermally in the same manner. The endpoint of the injection was subepidermal wheal-like swelling of the skin. The iBXTA injected side was assigned randomly for each volunteer. Independent researcher dispensed either iBXTA or normal saline. The iBTXA and normal saline were in the same form of syringe and allocated by the independent researcher. The volunteers and researchers were blinded until the final evaluation were done.

Assessment criteria

The volunteers were instructed to visit the clinic on the injection day and at weeks 2, 4, 8, and 12 after injection. At each visit, a medical photograph was taken, and pore sizes and wrinkles of the infraorbital area were evaluated with a facial analyzer (Mark-Vu®; PSI PLUS, Deajeon, Korea). Pore sizes were measured as a percentage of skin surface covered. Sebum secretions from both cheeks were evaluated using a sebumeter (DermaLab®; Cortex Technology, Hadsund, Denmark) after 30 minutes of facial washing at each visit. Improvement or aggravation in skin texture was evaluated by both volunteers and clinicians on a numeric scale from −4 to +4 (−4: severe aggravation, +4: marked improvement) at each visit (Table 1). After photographic review, the wrinkle score of the nasolabial fold was graded on a 5-point scale (from 0 to 5) introduced by

Table 1. The degree of improvement or aggravation in skin texture evaluated by the participants and clinicians

−4	−3	−2	−1	0	+1	+2	+3	+4
Severe aggravation	Moderate to severe aggravation	Moderate aggravation	Mild aggravation		Mild improvement	Moderate improvement	Moderate to marked improvement	Marked improvement

Based on the article of Kapoor et al. Dermatol Surg 2010;36 Suppl 4:2098-2105[12].

Lemperle et al[14].

Statistical analysis

The Wilcoxon signed rank test was used to compare pore sizes, wrinkles of the infraorbital area and nasolabial fold, and sebum secretion between pre- and post-treatment. The Generalized Estimating Equation method was used to compare skin texture pre- and post-treatment as evaluated by volunteers and clinicians. The mean score obtained after iBTXA injection was compared with that after normal saline injection by the Wilcoxon signed rank test. All statistical analyses were conducted by two biostatistics specialists (SW Kim and HW Han). All data were analyzed using IBM SPSS Statistics ver. 22.0 (IBM Corp., Armonk, NY, USA). *p*-values of less than 0.05 were considered significant.

RESULTS

Demographics

Eighteen volunteers who visited our center from July 2020 to December 2020 were enrolled, and 15 completed the study. One volunteer was excluded from the study due to the side effect of facial palsy on the iBTXA injected side, and two volunteers were excluded due to loss of follow-up. None of the volunteers had any contraindications for BTXA injection. The demographics and baseline data of volunteers are shown in the Table 2. Fifteen volunteers with a mean age of 41 years (range, 30 to 54 years) were included in the analysis. There were not significant differences in the baseline characteristics between both injection sides.

Table 2. Baseline volunteer demographics and clinical data

Variable	Total	iBTXA	N/S
Mean age (yr)	41 (30~54)		
Sex (n)			
Male	10		
Female	5		
Pore (%)		53.6	53.1
Sebum secretion (%)		3.2	3.13
Wrinkle			
Nasolabial fold (scale)		1.9	1.9
Infraorbital area (%)		26.0	26.4

Pore (%) was evaluated using a facial analyzer (Mark-Vu®; PSI PLUS, Deajeon, Korea). Sebum secretion was measured using a sebumeter. Nasolabial fole wrinkle was graded using a 5 point scale (from 0 to 5). Infrorbital area (%) indicates the percentage of area of infraorbital wrinkles to the infraorbital area based on a facial analyzer. iBTXA: incobotulinum toxin A, N/S: normal saline.

Changes in pore size and area

In the iBTXA injected side, the percentage of skin surface covered by pores improved significantly from 53.60%±8.34% to 51.53%±7.70% at two weeks after injection (p=0.03, Table 3). The normal saline-injected side exhibited improvement in pores from 53.07%±8.96% to 52.20%±7.19%, and the difference between the iBTXA injected side and the control side was not significant. Pore size also improved from four weeks to 12 weeks with either iBTXA or normal saline injection, and the changes compared with baseline were not significant. The degree of improvement was not significant between iBTXA and normal saline injections at any time point.

Changes in the nasolabial fold

The wrinkle scale of the nasolabial fold in the iBTXA injected side exhibited significant improvement at each assessment. As seen in Table 3, the assessment at week 4 showed the largest improvement from 1.87±1.25 to 0.93±1.33 (p<0.01). At eight weeks after injection, the wrinkle scale of the nasolabial fold had returned gradually to baseline, as seen in Fig. 1. At week 12, there was a significant difference of improvement between iBTXA and normal saline injections.

Infraorbital wrinkles and sebum secretion

Wrinkles of the infraorbital area exhibited slight but not significant improvement on both the iBTXA and normal saline injected sides (Table 3). The sebumeter showed improvements at weeks 4, 8, and 12, but none was statistically significant on either side. At week 2, there was a slight increase in sebum secretion (Table 3).

Improvement of skin texture based on volunteer satisfaction

Sixty percent of the volunteers (9/15) reported that their skin texture improved on the iBTXA injected side, while 26.7% of volunteers (4/15) reported that their skin texture improved on the normal saline injected side at two weeks after injection (Table 4). This improvement was only statistically significant in the iBTXA injected side (p=0.02). At four weeks

Table 3. Changes in pore, wrinkles and sebum secretion from pre-treatment to post-treatment with iBTXA and normal saline injection

Variable	iBTXA		Normal saline (N/S)		iBTXA vs. N/S
	Mean±SD	*p*-value*	Mean±SD	*p*-value *	*p*-value
Pore (%)					
Week 0	53.60±8.34		53.07±8.96		
Week 2	51.53±7.70	0.03	52.20±7.19	>0.99	0.41
Week 4	52.00±7.79	0.34	52.00±7.79	>0.99	0.92
Week 8	52.07±7.94	0.52	51.73±7.44	0.98	>0.99
Week 12	52.67±7.33	>0.99	52.60±7.16	>0.99	>0.99
Wrinkle of nasolabial fold (Wrinkle score)					
Week 0	1.87±1.25		1.87±1.36		
Week 2	1.07±1.39	0.02	1.60±1.18	0.50	0.22
Week 4	0.93±1.33	<0.01	1.73±1.22	>0.99	0.05
Week 8	1.00±1.31	<0.01	1.73±1.22	>0.99	0.06
Week 12	1.13±1.36	0.02	1.73±1.33	>0.99	0.03
Wrinkle of infraorbital area (%)					
Week 0	26.00±4.39		26.40±4.79		
Week 2	24.33±3.50	0.15	25.73±3.24	>0.99	0.64
Week 4	24.33±3.89	0.08	25.67±3.56	>0.99	0.13
Week 8	25.00±3.02	0.82	25.40±3.72	>0.99	>0.99
Week 12	25.87±3.72	>0.99	25.80±3.71	>0.99	>0.99
Sebum secretion (%)					
Week 0	3.20±8.52		3.13±8.48		
Week 2	4.07±7.65	>0.99	4.40±9.52	>0.94	>0.99
Week 4	1.73±4.27	>0.99	2.33±3.66	>0.99	0.84
Week 8	2.00±3.64	>0.99	2.53±5.26	>0.99	>0.99
Week 12	1.33±3.11	>0.99	1.67±3.37	>0.99	>0.99

p-value is corrected by Bonferroni correction. iBTXA: incobotulinum toxin A, SD: standard deviation. *Compared to week 0.

after injection, 86.7% of volunteers (13/15) reported that their skin texture improved on the iBTXA injected side ($p<0.01$), while 33.3% of volunteers (5/15) reported improvement on the other side. A more than moderate degree of improvement on the iBTXA injected side was reported in 73.3% of volunteers (11/15). At eight weeks after injection, 66.7% of the volunteers (10/15) reported improvement in skin texture on the iBTXA injected side ($p<0.01$). At 12 weeks after injection, 40.0% of the volunteers (6/15) reported improvement on skin texture on the iBTXA injected side, but the difference from the control side was not significant ($p=0.13$). On the other hand, several volunteers reported that their skin texture improved on the normal saline-injected side, but the scores were not significantly different from baseline at any week. Only at four weeks after injection was a significant difference noted between the iBTXA and normal saline injected sides ($p<0.01$).

Improvement of skin texture as evaluated by the clinician

Skin texture evaluated by the clinician exhibited significant improvement on the iBTXA injected side at weeks 2, 4, and 8 ($p<0.01$; Fig. 1, Table 4). At two and four weeks after injection, 93.3% of volunteers (14/15) showed improvement. At eight weeks after injection, 86.7% of volunteers (13/15) showed improvement, but the degree of improvement decreased from that at four weeks. At 12 weeks after injection, 46.7% of volunteers (7/15) exhibited improvement of skin texture, but the change was not significant ($p=0.06$). On the normal saline-

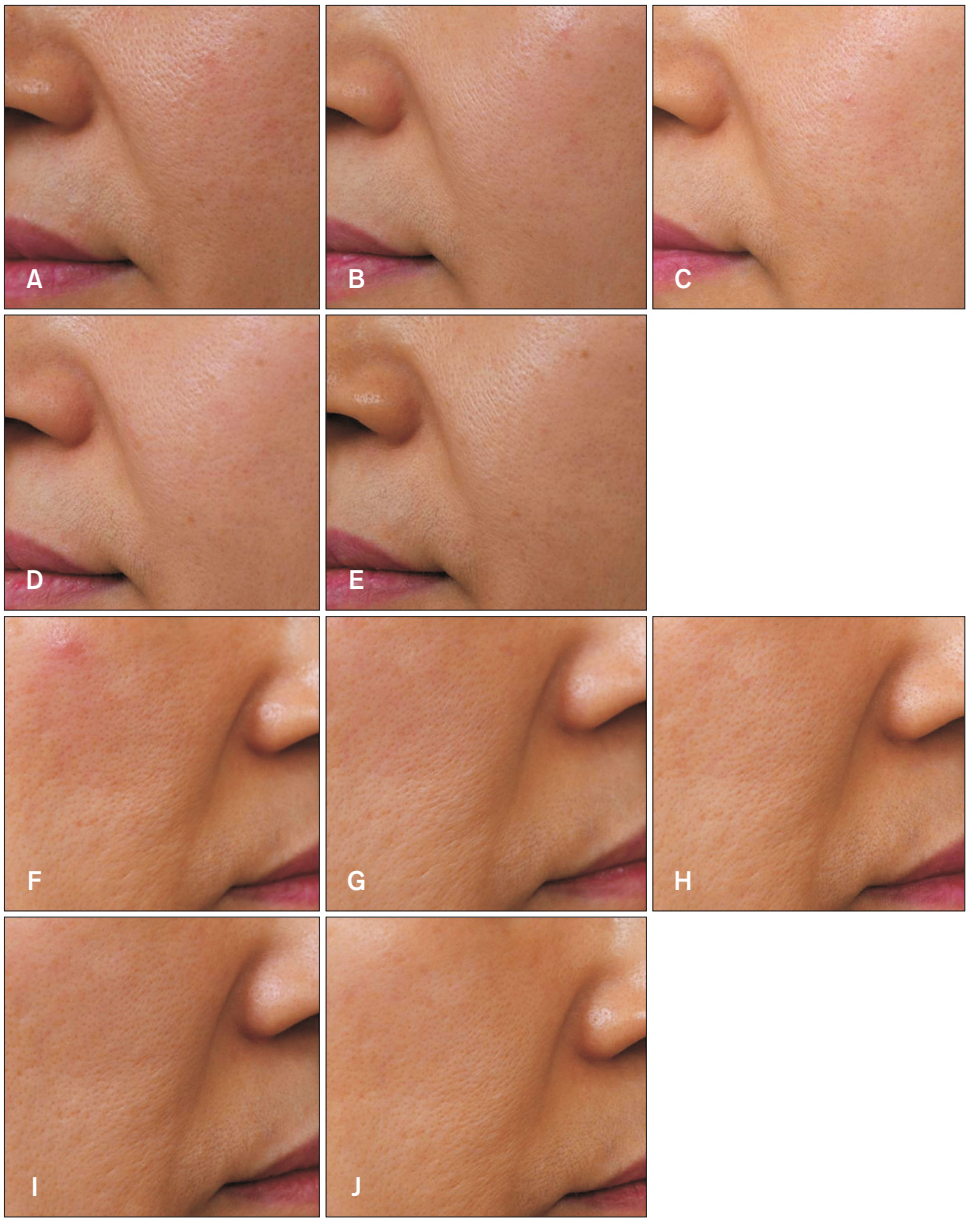

Fig. 1. A 51-year-old female volunteer was injected incobotulinum toxin A (iBTXA) on the left cheek and normal saline on the other side. After iBTXA injection, her skin texture appeared to be tenser and showed fewer furrows and pores compared with pretreatment, and it returned gradually at week 12. On the other hand, opposite side of cheek showed no changes. iBTXA injected side: (A) pretreatment, (B) 2 weeks after, (C) 4 weeks after, (D) 8 weeks after, and (E) 12 weeks after. Normal saline injected side: (F) pretreatment, (G) 2 weeks after, (H) 4 weeks after, (I) 8 weeks after, and (J) 12 weeks after.

injected control side, only one volunteer showed improvement of skin texture at weeks 2 and 4, at which injections of iBTXA and normal saline produced significant differences in skin texture (p<0.01). At weeks 8 and 12, no one showed improvement in skin texture on the normal saline injected side. Therefore, comparisons between iBTXA and normal saline injected sides could not be performed.

Adverse effects and events

After a total of 30 U of iBTXA was injected intradermally in the cheek of one volunteer, she complained of facial palsy. The volunteer was excluded from the study and underwent injection of iBTXA on the other side of the face for balance at 2 weeks after the initial injection. One month later, the uncomfortable facial palsy had improved. Therefore, we reduced the total injection dose for all other volunteers from 30 U to less than 20 U. None of the other volunteers experienced serious adverse effects, such as facial palsy, allergic reaction, or severe paralysis of muscle adjacent to the point of injection during or after the study. The most common adverse effects were pain and stinging sensation during the injection. However, pain was tolerable and resolved in all volunteers within 30 minutes.

Table 4. Changes in skin texture evaluated by participants and clinicians in numeric scale

Variable	iBTXA						Normal saline (N/S)						iBTXA vs N/S
	0	1	2	3	4	*p*-value*	0	1	2	3	4	*p*-value*	*p*-value
Improvement of skin texture evaluated by participants													
Week 2	6 (40.0)	3 (20.0)	4 (26.7)	2 (13.3)	0 (0.0)	0.02	11 (73.3)	2 (13.3)	2 (13.3)	0 (0.0)	0 (0.0)	0.5	0.032
Week 4	2 (13.3)	2 (13.3)	6 (40.0)	4 (26.7)	1 (6.7)	<0.01	10 (66.7)	4 (26.7)	1 (6.7)	0 (0.0)	0 (0.0)	0.25	<0.01
Week 8	5 (33.3)	3 (20.0)	3 (20.0)	4 (26.7)	0 (0.0)	<0.01	10 (66.7)	4 (26.7)	1 (6.7)	0 (0.0)	0 (0.0)	0.25	0.06
Week 12	9 (60.0)	2 (13.3)	3 (20.0)	1 (6.7)	0 (0.0)	0.13	13 (86.7)	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)	>0.99	0.23
Improvement of skin texture evaluated by clinicians													
Week 2	1 (6.7)	2 (13.3)	10 (66.7)	2 (13.3)	0 (0.0)	<0.01	14 (93.3)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	>0.99	<0.01
Week 4	1 (6.7)	1 (6.7)	10 (66.7)	3 (20.0)	0 (0.0)	<0.01	14 (93.3)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	>0.99	<0.01
Week 8	2 (13.3)	7 (46.7)	3 (20.0)	3 (20.0)	0 (0.0)	<0.01	15 (100.0)	0 (0.00)	0 (0.0)	0 (0.0)	0 (0.0)	-	-
Week 12	8 (53.3)	5 (33.3)	2 (13.3)	0 (0.0)	0 (0.0)	0.06	15 (100.0)	0 (0.00)	0 (0.0)	0 (0.0)	0 (0.0)	-	-

Values are presented as number (%) unless otherwise indicated. iBTXA: incobotulinum toxin A. *Compared to week 0.

No other serious adverse events were reported.

DISCUSSION

The BTXA is composed of 150 kD neurotoxin and the rest of complexing proteins. The neurotoxin is the very part of exerting clinical effect and the role of complexing proteins is not clear[15]. However complexing proteins have higher protein load in the BTXA and it may concerned with neutralizing antibodies[5,15,16]. Compared with other classic BTXA, iBTXA has the lowest complexing proteins, and may have least chances related with neutralizing antibodies, which may be responsible for the secondary treatment failure in the setting of long-standing repeated BTXA administrations[15,16]. Intradermal injection of BTXA is usually repeated at weeks intervals, which is a shorter period compared with the conventional intramuscular injection of BTXA. From this point of view, iBTXA may be more suitable for the intradermal injection. In addition, previous studies demonstrated that iBTXA has the equal clinical strength and safety compared with classic onabotulinum toxin type A[17-19].

There have been several reports on the efficacy and safety of intradermal injection of conventional BTXAs (onabotulinum toxin A and abobotulinum toxin A)[2,4,13,20-22]. Among them, Sapra et al.[4] compared the effects on skin texture and midface lift of intradermal and intramuscular injections of the two conventional BTXAs and showed no statistically significant differences between two BTXAs. However, there have been no previous studies regarding to intradermal injection of iBTXA until now. To the best of our knowledge, this is the first report on the efficacy and safety of intradermal injection of iBTXA.

This study demonstrated that intradermal BTXA injection, with iBTXA, improves skin texture and wrinkles of the nasolabial fold beyond the effect of simple needle pricking. Recently published report demonstrated the effect of BTXA intradermal injection compared with normal saline as a control using the split face study[22]. The study design appeared to be the almost same as ours, but the injection method was totally different. We performed intradermal injections on the whole cheeks at 1cm interval like the previous reports[2,4,13,20,21]. However, Atwa et al.[22] conducted the injection at fifteen points on one side of face (four points arranged in two rows over the frontalis muscles below the hairline, two points at the temporal area along the hair line, one centimeter apart, points injected at one centimeter intervals in the crow's feet area, and another two points in the front of the ear at the level of the tragus and auricle. Another four points along the mandibular line).

Based on the working mechanism of BTXA[1], the actual effect of intradermal injection of BTXA in skin remains unknown. However, intradermal injection of BTXA has been performed widely with success in the real world without any serious adverse events like facial palsy, a major limitation and drawback of BTXA injection on the face[4,20,21]. Our previous study[3] also showed that the anti-wrinkle effect of BTXA on the forehead did not significantly differ between intramuscular and intradermal injections. However, side effects such as ptosis oc-

curred at a much lower rate in the intradermal injection side[3].

A previous report by Zhu et al.[21] demonstrated that 30U of BTXA intradermal injection into one side of the face exhibited the clinical benefit of facial rejuvenation and no specific adverse effects. However, clinicians commonly use lower total doses of BTXA intradermal injection. We initially conducted intradermal injection of iBTXA at a total of 30 U into one side of the face, as in a previous report[21]. However, this produced facial palsy on the injected side. Therefore, we lowered the total dose of iBTXA injection from 30 U to less than 20 U.

In this study, definite improvement of skin texture and the nasolabial fold was observed with no significant improvements of wrinkles of the infraorbital area and sebum secretion. For pores, significant positive effects were observed only at two weeks after injection. However, no significant differences between iBTXA and normal saline injections were observed, even though the degree of improvement of pore size was higher in the iBTXA injected side. One explanation might be the small number of volunteers enrolled in this study. Another possible reason was that edema caused by multiple injections lead to short-term improvement of pore size.

Regarding sebum secretion and pores, several studies reported that intradermal injection of BTXA could play a significant role through its blockade of cholinergic signaling and its neuromodulatory effects on the arrector pili muscle and local muscarinic receptors within the sebaceous gland[1,13,20]. On the other hand, Sapra et al.[4] reported that intradermal injection of BTXA did not provide the significant reductions of pore size and sebum secretion as in our results. This discrepancy is believed to be due to the heterogeneity of the volunteers and injected doses. While one of these studies[20] included volunteers with oily skin, the other[1] did not. One of our volunteers, who exhibited the highest pore size and the second highest sebum secretion, showed positive effects on sebum secretion and pore size after iBTXA injection through facial analyzer and sebumeter, pore from 67% to 56% and sebumeter from 38% to 4%. Previous studies demonstrated that human skin sebaceous glands *in vivo* and sebocytes *in vitro* express nicotinic acetylcholine receptor α7, and that acetylcholine increased lipid synthesis in a dose-dependent manner as well as the differentiation of sebocytes[1,23]. When sebocytes were incubated with α-bungarotoxin, a competitive nicotinic acetylcholine receptor antagonist, acetylcholine failed to up-regulate lipid synthesis[1,23]. Based on these findings, BTXA might play a role in human sebaceous gland biology affecting sebum secretion and pore size. Although the present study failed to show significant improvement of pore size after intradermal iBTXA injection, the pore size tended to decrease more on iBTXA injected side than normal saline injected side. The enrollment of more oily patients might have yielded the statistically significant results.

In this study, significant improvement in wrinkles of the nasolabial fold and skin texture as evaluated by volunteers and clinicians were observed. Several investigators have proposed mechanisms including paralysis of depressor muscles, increase in collagen synthesis and fibroblast contraction[2,24]. Previous study reported greater collagen deposition after BTXA injection through Masson trichrome staining upon histological examination[2]. In human fibroblasts, contraction of fibroblasts was observed in an *in vitro* study, possibly explaining the skin lifting effect of intradermal BTXA injection[24]. Another plausible explanation that BTXA intradermal injection improves skin texture is the transient lymphatic insufficiency caused by BTXA and relaxation of smooth muscles around vessels and lymphatics. This may stimulate the collagen synthesis via inflammation. Improvement was noted at 4 weeks after iBTXA injection and gradually decreased at 2 to 3 months later in the present study. This timeline suggested that initial improvement of skin texture and wrinkles appeared to be related with transient edema caused by iBTXA and subsequent stimulation of collagen synthesis kicked off by inflammation could take place.

Possible demerits of intradermal BTXA injection compared to intramuscular BTXA injection are more severe pain because of multiple needle pricks and shorter duration of the toxin effects. However, the pain can be alleviated by preprocedural topical anesthetics or ice pack application. While further studies are needed, the effects on nasolabial wrinkles were maintained until week 12, suggesting a small difference in the duration of the effect between intradermal and intramuscular injections. Moreover, no more than 20 U of iBTXA intradermal injection on one of the cheeks did not induce noticeable facial palsy. Therefore, intradermal injection of iBTXA has value as a cosmetic procedure when the appropriate dose is applied.

The limitation of this study is the small sample size, which could have affected the significance of the effects of BTXA injection and might limit the observation of possible side effects. Therefore, future studies with larger sample sizes are needed.

In conclusion, this study demonstrates that intradermal injection at 1 cm interval with iBTXA of less than 20 U on one cheek provide beneficial effects on wrinkles of the nasolabial fold and skin texture overcoming the skin prick effect without obvious side effects. Further studies are necessary with larger numbers of volunteers and adjustable doses for intradermal injections of iBTXA as well as the direct comparison between other BTXAs.

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Two biostatistics specialists (SW Kim and HW Han) were involved in analysis of the data.

CONFLICTS OF INTEREST

Medytox provided the research funding for this study. However, it did not participate in the study design, data collection, analysis, or writing of the manuscript.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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