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What is This?
Skin Biomechanical Changes after Injection of Onabotulinum Toxin A: Prospective Assessment of Elasticity and Pliability

James P. Bonaparte, MD, MSc, FRCSC¹, and David Ellis, MD, FRCSC²

Abstract

Objective. This study aimed to test the hypothesis that the administration of onabotulinum toxin A will result in an increase in skin pliability and elasticity.

Study Design. A prospective case series with planned data collection in which patients were treated with onabotulinum toxin and assessed at baseline, 2 weeks post-injection, and 2 months post-injection.

Setting. A private cosmetic surgery clinic associated with a tertiary academic hospital.

Subjects and Methods. Forty women were prospectively enrolled to receive onabotulinum toxin A into their glabella, forehead, and lateral orbit. Outcomes were assessed at baseline, 2 weeks posttreatment, and 2 months posttreatment using the Cutometer MPA 580. Skin pliability (Uf) and the elastic recoil (Ua/Uf) were recorded as the 2 primary outcome measures.

Results. There was a significant effect of onabotulinum toxin on skin elasticity (f = 47.8, \(P = .001\)) with a mean (+/− SE) increase in elastic recoil of 20% (4.4%) for the glabellar region (\(P < .001\)) and 18% (4.0%) for the lateral orbit (\(P < .0001\)). There was a significant effect of the treatment on skin pliability (f = 46.9, \(P < .001\)) with a mean (+/− SE) increase of 26% (5.4%) for the lateral orbit (\(P = .001\)) and 52% (8.3%) for the glabellar region (\(P < .001\)).

Conclusion. Injection of onabotulinum toxin into the lateral orbital, forehead, and glabellar regions results in skin that has increased pliability as well as increased elastic recoil. Although this study demonstrates the positive effect of onabotulinum toxin on biomechanical parameters, it is unclear what specific histological changes are occurring within the skin.

Keywords

biomechanics, botulinum toxin A, cosmetic surgery, skin

Introduction

From a biomechanical perspective, youthful skin has 3 important features: strength, pliability (or compliance, ie, the ability to stretch), and elasticity (or resilience, ie, the ability to recoil). As skin ages, alterations occur in these biomechanical properties. These changes can be the result of both intrinsic and extrinsic factors. Intrinsic changes primarily involve those related to genetics, whereas extrinsic changes are primarily a result of sun exposure. Although both contribute to aging, it is thought that only 3% to 20% of aging is related to intrinsic factors, whereas the majority is due to more preventable extrinsic processes.

Both aging and ultraviolet (UV) radiation exposure lead to an increase in elastase activity, resulting in a reduction in skin elasticity and alterations in its tensile strength. The loss of skin elasticity is the most prominent change in aging skin. These changes precede the telltale signs of skin damage—wrinkles.

Numerous treatments have been developed to treat the signs of aging skin. Onabotulinum toxin type A (Botox; Allergan Inc, Irvine, California, USA) is a commonly used biological medication for a variety of medical and cosmetic indications. Although very effective in reducing dynamic

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facial wrinkling, the vast majority of studies assessing the
efficacy of onabotulinum toxin focus on short-term results.12-15

Recently, a number of articles have noted that onabotulinum
toxin appears to result in a progressive reduction in
long-standing facial rhytides.16,17 It remains unclear why
these progressive and long-term changes in wrinkle levels
are occurring. One theory suggests that it is a result of a
learned response, such that patients unlearn the ability to
recruit their facial frowning muscles, resulting in reduced
glabellar motion. A second theo... of the injection of
onabotulinum toxin may have a
direct effect on the skin at a histologic or intrinsic level.

To help identify potential intrinsic changes occurring in
facial skin after onabotulinum toxin treatment, it would be
ideal to use subjective measures of skin mechanics such as
the Cutometer MPA 580 (Courage + Khazaka Electronic,
Cologne, Germany) to determine, in particular, if an injec-
tion of onabotulinum toxin results in biomechanical changes
that are opposite those that one would expect with aged or
wrinkled skin.

Therefore, the purpose of this study is to test the hypoth-
esis that an injection of a standard cosmetic dose of onabo-
tulinum toxin into 3 sites on the face will result in an
increase in skin elasticity and pliability as measured by the
Cutometer at 2 months. As a secondary objective, this study
will assess the relative contribution of the viscoelastic and
pure elastic resistance within the skin after the injection of
the onabotulinum toxin.

Material and Methods

Patient Population

All patients presenting to the facial plastic surgery clinic for
an injection of onabotulinum toxin over the glabella, forehead,
and lateral orbit were approached for enrollment. Patients
were enrolled consecutively over the course of 3 months
between August and October 2012. The clinic is associat-
ed with the Department of Otolaryngology–Head and Neck
Surgery at the University of Toronto (Toronto, Canada). The
clinic performs between 1000 and 1200 botulinum injections
a year. All research followed the Helsinki declaration, and
subjects signed a written informed consent approved by our
institution prior to enrollment. This study was approved by
the research ethics board for the University of Toronto.

Exclusion Criteria

Patients were excluded if they met any of the following cri-
eria: (1) pregnant or breastfeeding, (2) a medical comorbidi-
ty resulting in a contraindication to onabotulinum toxin, (3)
scars or other anomalies over the measurement sites, (4) any
dermatological conditions underlying the measurement site, (5)
onabotulinum toxin injection within 6 months of the ini-
tial visit, (6) if patients were undergoing concomitant or
planned skin tightening (both laser or surgical), and (7) any
other facial laser procedures during the treatment period or
within 2 months prior to the initial treatment.

Onabotulinum Toxin A Injection

Onabotulinum toxin A was used for all patients. Each 200-
unit vial of onabotulinum toxin was mixed with 2 cc of
0.9% sodium chloride with preservatives. Prior to the injec-
tion, each patient was assessed by a second physician not
involved in data collection who determined the dose
required to achieve maximum benefit of the treatment.
Doses followed the 2008 Consensus Recommendations for
botulinum toxin injections.18

A 30-gauge needle was used for all injections. One injec-
tion was performed over the procerus, 3 injections were per-
formed along the corrugator bilaterally, and 3 were per-
formed along the orbicularis bilaterally as described by
Carruthers et al.19 No additional injections were performed
after initiation of the study.

Cutometer Instrumentation and Settings

A Cutometer MPA 580 along with the accompanying soft-
ware (Cutometer MPA Q) was used for all patients.

All patients were tested using the time-strain mode
(mode 1). For each trial, a 3-second active suction followed
immediately by a 3-second suction-off relaxation period
was used with a setting of 400 mbar (40 kPa) of suction.
This 6-second on-off period will be referred to as 1 cycle in
this article.

Data Collection

Data were collected at 3 points: pretreatment, 2 weeks post-
treatment, and 2 months posttreatment. The experimental
protocol was the same for each period. The same investiga-
tor collected data for all periods.20 This investigator was
blinded to the results of the testing until all data collection
was completed.

All testing was performed in a humidity- and temperature-
controlled environment. The temperature was maintained at
23°C with a relative humidity of 35%.

Three testing sites on the patient’s face (treated sites) and
1 on the forearm (control site) were identified. All testing
was completed with the patient at repose. Only 1 side of the
face, determined during the intake session by coin toss, was
tested.

Testing site 1 was at the glabella, defined as the point
halfway between the medial brow above the nasion. Site 2
was the supraorbital point, defined as the point correspon-
ding to a vertical line dropped from the lateral limbus of the
eye at a right angle to the Frankfort Horizontal 1.5 cm
above the orbital rim above the brow. This site was chosen
as the approximate point at which the corrugator superci-
lous inserts into the skin.21 Site 3 was the lateral orbit
defined as 1 cm lateral to the orbital rim at the mid-
pupillary line. The patient’s forearm skin was defined as a
distance of 10 cm measured from the proximal volar wrist
crease in the mid-aspect of the patient’s right forearm.
post-hoc testing was used to assess for individual differences assessed as potential covariates in the model. Tukey’s test was used to determine if there was a significant effect of both time (baseline, 2 weeks, and 2 months), and the interaction between the variables. Both total dose and age were normally distributed, and thus transformations were performed. A sample size calculation for a 1-way ANOVA with 4 levels was completed. Assuming that $P = .025$ with a mean difference between groups of 10% with a standard deviation (SD) of 10% at a power of 80%, a minimum sample size of 28 patients was required. The standard deviation of 10% was estimated by pilot testing as well as previously published research.10

To capture the measurement, the investigator placed the probe on the site at the marked location at a 90° angle to the skin. After 1 measurement cycle was completed, the probe was removed from the patient’s skin and a 15-second rest was administered. This rest was performed to limit the effect of elastic hysteresis. Each site was tested 3 times with the median value being used for the final data analysis.

Outcome Measures

There were 2 primary outcome measures collected for this study: pliability ($U_f$) and overall elasticity ($U_a/U_f$) (Figure 1).

When the skin is being stretched, there are 2 components resisting lengthening of the skin: the pure elastic resistance ($U_e$) and the viscoelastic resistance ($U_v$). As a secondary outcome measure, the ratio of the $U_v/U_e$ was calculated.

Other measurements recorded by the Cutometer include the $U_a$, $U_e$, $U_v$, $U_r$, $U_r/U_f$, $U_a/U_f$, and $U_f-U_a$ (Figure 1).20

Statistics and Data Analysis

Demographic data including age, sex, Fitzpatrick skin type, overall total dose (units), and Glagou Facial Wrinkle Score22 were recorded for each patient.

Both primary outcomes were tested to determine if they differed significantly from a normal distribution using the Anderson–Darling test. Any distribution with a $P$ value < .05 was considered to be significantly different from a normal distribution, and thus transformations were attempted using a Johnson transformation.

For each primary and secondary outcome measure, a general linear model analysis of variance (ANOVA) with repeated measures was used to determine if there was a statistically significant difference between site (3 sites), time (baseline, 2 weeks, and 2 months), and the interaction between the variables. Both total dose and age were assessed as potential covariates in the model. Tukey’s post-hoc testing was used to assess for individual differences within the ANOVA. Data were analyzed using Minitab 15 (Minitab Inc, State College, Pennsylvania, USA).

Since 2 primary outcome measures were collected, a Bonferroni adjustment was performed; thus, statistical significance was defined as a $P < .025$.

Sample Size Calculation

A sample size calculation for a 1-way ANOVA with 4 levels was completed. Assuming that $P = .025$ with a mean difference between groups of 10% with a standard deviation (SD) of 10% at a power of 80%, a minimum sample size of 28 patients was required. The standard deviation of 10% was estimated by pilot testing as well as previously published research.10

Results

Forty-three patients enrolled in the study. Of those, 3 did not return for follow-up and thus were excluded from the data analysis. The mean (SD) age of patients was 52.2 (9.5) years with 100% of patients being female. The mean (SD) total dose for all 3 injection sites was 46.8 (11.7) units. The mean (SD) dose for each side was 8.6 (4.9) units for each lateral orbit side, 12.2 (2.9) units for the corrugator, and 5.3 (1.29) units for the midline procerus injection.

Both pliability ($U_f$) ($P = .16$) and elastic recoil ($U_a/U_f$) ($P = .10$) data were not significantly different from a normal distribution.

For pliability ($U_f$), there was a significant effect of time ($f = 47.8, P < .001$) and location ($f = 43.55, P < .001$) with no significant interaction ($f = 1.06, P = .38$) in the ANOVA model ($s = 0.20, R^2 = 53.05\%$) (Table 1, Figure 2). For elastic recoil, there was a significant effect of time ($f = 46.9, P < .001$) and location ($f = 4.6, P = .01$) with no significant interaction ($f = 1.74, P = .14$) in the ANOVA model ($s = 0.11, R^2 = 46.4\%$) (Figure 3, Table 2). Neither age nor dose of onabotulinum toxin was significant as a covariate in either model ($P > .60$).

For pliability, post-hoc testing indicated that there was no significant change between baseline and 2 weeks for any site. There was, however, a significant increase at all 3 sites between baseline and 2 months ($P < .001$) (Table 1). For elastic recoil, there was no significant change between baseline and 2 weeks for both the lateral orbit ($P = .67$) and the supraorbit ($P = .92$). There was a significant increase in elastic recoil at the glabella at 2 weeks ($P = .001$). For all sites, there was a significant increase in elastic recoil between baseline and 2 months ($P < .002$) (Table 2).

When assessing the ratio of the elastic component versus the viscoelastic component of skin ($U_v/U_e$), there was a significant effect of both time ($P < .001, f = 19.57$) and location ($P < .001, f = 40.05$) (Figure 4). Specifically, between baseline and 2 months after botulinum treatment, there is a significant reduction in the ratio of $U_e$ (elastic component) to $U_v$ (viscoelastic component) (ANOVA; $s = 0.1114, R^2 = 44.83$). Data for all Cutometer parameters are presented in Table 3.
The forearm (the control site) did not demonstrate any significant change over time for pliability ($P = .15$, $R^2 = 59.85\%$), elastic recoil ($P = 0.11$, $R^2 = 66.86\%$), or the ratio of Uv/Ue ($P = 0.34$, $R^2 = 25.87\%$).

### Discussion

The results of this study confirm our initial hypothesis, specifically, that the injection of onabotulinum toxin A for facial wrinkles results in an increase in both pliability and elastic recoil of the surrounding skin. This effect was more than anticipated, as the mean increase in elasticity was nearly 20% at the 2-month period.

When viewing a stress-strain curve of skin, there are 3 primary phases. Phase 1 is the purely elastic component in which the resistance is minimal and provided solely by the elastic fibers as they elongate (defined as Ue in the current study). The second phase, the viscoelastic phase, is the transition in which the elastic fibers offer resistance while fluid within the skin begins to adjust. The final phase is the constant creep phase in which resistance is due primarily to collagen fibers. The combination of phases 2 and 3 was considered the viscoelastic phase (Uv) in the current study, as the Cutometer provides data for these phases together. As time from onabotulinum toxin treatment increased, there was an increase in phase 1 (pure elastic component) and a decrease in phases 2 and 3 (viscoelastic) within the skin.

One possibility for the increase in phase 1 (Uf) may be that onabotulinum toxin induces the production of a more organized dermal elastin fiber network, allowing for less initial resistance to stretch. The reduction in phases 2 and 3 typically occurs with increased collagen production, resulting in an increase in resistance to deformation once the elastic fibers have reached their maximal extension. In addition to this, the increased elastic recoil (Ua/Uf) of the skin suggests that the tissue now has an increase in its stored elastic potential energy, thus when released, the skin has the ability to retract to a greater extent as demonstrated by the increase in elastic recoil. These changes produce skin that has the characteristic features more consistent with youthful skin.

What remains unclear is why the wrinkles fade with time. The paralysis of the muscle provided by onabotulinum toxin may lead to a reduction in repetitive skin folding at future wrinkle sites as well as a reduction in chronic stress applied by the facial muscles. Over time, the elastin and collagen weaken at these sites. Based on the current study,

### Table 1. Results of the Analysis of Variance for Pliability (Uf).

<table>
<thead>
<tr>
<th>Location</th>
<th>Time</th>
<th>Mean % Change From Baseline</th>
<th>95% CI Lower Bound</th>
<th>95% CI Upper Bound</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P Value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral orbit</td>
<td>2 wk</td>
<td>0.74</td>
<td>-13.676</td>
<td>12.196</td>
<td>.243</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>2 mo</td>
<td>26.98</td>
<td>13.693</td>
<td>40.267</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Supraorbit</td>
<td>2 wk</td>
<td>5.75</td>
<td>-7.013</td>
<td>18.513</td>
<td>.526</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>2 mo</td>
<td>33.28</td>
<td>19.9904</td>
<td>46.5696</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Glabella</td>
<td>2 wk</td>
<td>14.54</td>
<td>0.313</td>
<td>28.767</td>
<td>.044</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>2 mo</td>
<td>50.38</td>
<td>35.5629</td>
<td>65.1971</td>
<td>&lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

<sup>a</sup>P value represents a comparison of individual measurement time to pretreatment.

<sup>b</sup>Comparison of 2 weeks to 2 months.
it is likely that the effect of onabotulinum toxin on skin is more complex than simple muscle paralysis.

If onabotulinum toxin does have a direct effect on the skin, one would expect an intradermal injection to result in similar changes to those noted in this study. Chang et al\textsuperscript{27} assessed the effect of intradermal onabotulinum toxin on the skin of the cheek. The authors noted a significant reduction in cheek wrinkling on the onabotulinum toxin-treated side with no change on the side treated with saline injections.

Our team conducted a pilot study in our lab in 2 patients using a similar technique to Chang et al.\textsuperscript{27} Over the course of 16 weeks, there was a significant reduction in facial wrinkling with a progressive 20% increase in skin elasticity as measured by the Cutometer on the treated side compared to the untreated side.

The question as to how onabotulinum toxin results in skin tightening remains elusive. A study by Oh et al\textsuperscript{26} assessed the effect of onabotulinum toxin on human fibroblasts. At both 36 hours and 48 hours after exposure, there was a significant increase in pro-collagen, Col 1A1, and Col 1A2 and a reduction in MMP9 in a dose-dependent manner compared to controls. A second study confirmed a number of these findings.\textsuperscript{28}

If onabotulinum toxin is interacting with fibroblasts, the mechanism remains unclear. However, 1 study identified an acetylcholine receptor on fibroblasts.\textsuperscript{29} Another study\textsuperscript{25} noted that the c-terminal binding domain region of

**Table 3. Raw Results for all Cutometer Data.**

<table>
<thead>
<tr>
<th>Location</th>
<th>Lateral Orbit</th>
<th>Glabella</th>
<th>Supraorbital</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>2 Months</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>R0 (Uf), mm</td>
<td>0.94</td>
<td>0.29</td>
<td>1.16</td>
</tr>
<tr>
<td>R1 (Uf-Ua), mm</td>
<td>0.27</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>R2 (Ua/Uf), %</td>
<td>0.70</td>
<td>0.15</td>
<td>0.88</td>
</tr>
<tr>
<td>R3, mm</td>
<td>0.95</td>
<td>0.29</td>
<td>1.16</td>
</tr>
<tr>
<td>R4, mm</td>
<td>0.26</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>R5 (Uf/Ue), %</td>
<td>0.43</td>
<td>0.13</td>
<td>0.48</td>
</tr>
<tr>
<td>R6 (Uv/Ue), %</td>
<td>0.42</td>
<td>0.10</td>
<td>0.32</td>
</tr>
<tr>
<td>R7 (Ue/Uf), %</td>
<td>0.31</td>
<td>0.07</td>
<td>0.36</td>
</tr>
<tr>
<td>R8 (Ua), mm</td>
<td>0.68</td>
<td>0.33</td>
<td>1.03</td>
</tr>
<tr>
<td>Ue/Uf, %</td>
<td>70.88</td>
<td>4.66</td>
<td>75.99</td>
</tr>
</tbody>
</table>

**Abbreviation:** SD, standard deviation.
botulinum toxin A on human neural cells is homologous to fibroblast growth factors, thus a possible ligand to fibroblast growth factor receptor (FBGFR). These authors demonstrated that botulinum toxin A has a high affinity for these FBGFR on neuronal cells. Nevertheless, future investigations are required to answer these questions.

Although this current study provides evidence suggesting that the pliability and elasticity of the skin increases after onabotulinum toxin injection, there are limitations. As noted earlier, this study was not designed to determine the actual cause of the alteration in biomechanical properties of the skin. The hypotheses presented are purely speculative at this time. Ideally, histological confirmation would be performed to identify and confirm these changes.

This study also used a variety of doses individualized for each patient. Initially, the study design required all patients to have a standard dose for each site; however, the ethics of this were questioned, as individuals benefit from a treatment regime based on their particular anatomy and wrinkle pattern. The issue of variable dosing is particularly limiting if attempting to compare to other studies that used a single dose. However, it is important to note that the dose was not a significant covariate in the ANOVA model.

This study is the first to demonstrate an alteration in skin pliability and elasticity after treatment of onabotulinum toxin A for facial wrinkling. The changes occurring in patients’ skin appear to be the opposite of those associated with both the aging process and UV radiation exposure. Future studies are required to determine and quantify the histologic changes that are occurring.

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**Author Contributions**

James P. Bonaparte, project idea, data collection, preparation, analysis; David Ellis, project development, preparation, analysis.

**Disclosures**

**Competing interests:** Dr Bonaparte received an unrestricted educational grant from Allergan Canada. Dr Ellis received unrestricted funding, has spoken at meetings, and has served on the advisory board for Allergan Canada (Botox); Allergan USA–Research Grant; Cutera–Speaker.

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