Randomized Controlled Crossover Trial Comparing the Impact of Sham or Intranasal Tear Neurostimulation on Conjunctival Goblet Cell Degranulation

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• PURPOSE: The aim of the study was to investigate the effects of the Allergan Intranasal Tear Neurostimulator (ITN) on conjunctival goblet cell (GC) degranulation.
• DESIGN: A randomized, double-masked, placebo-controlled crossover trial.
• METHODS: A total of 15 subjects (5 normal and 10 dry eye) were enrolled in a 3-visit study consisting of 1 screening and 2 separate randomized-masked ITN treatments (sham extranasal or intranasal). Tear meniscus height (TMH) was measured by anterior segment optical coherence tomography before and after applications. Impression cytology (IC) was taken from the bulbar conjunctiva of the right eye for periodic acid–Schiff staining and from the left eye for MUC5AC mucin immunostaining at baseline and after each treatment. The ratio of degranulated to nondegranulated GCs was measured as a marker of secretion.
• RESULTS: In all participants, both inferior bulbar (IB) and temporal bulbar (TB) cytology specimens stained for MUC5AC revealed a significantly higher ratio of degranulated to nondegranulated GCs after the ITN (IB: 2.28 ± 1.27 and TB: 1.81 ± 1.01) compared to baseline (IB: 0.56 ± 0.55, $P = .015$) (TB: 0.56 ± 0.32, $P = .003$) and extranasal sham application (IB: 0.37 ± 0.29, $P = .001$) (TB: 0.39 ± 0.33, $P = .001$). When the same analysis was repeated in the dry eye or control groups, the ratio was significantly higher after ITN than the baseline ratio and ratio after extranasal application in both groups ($P < .05$). Moreover, although control subjects had a higher ratio of degranulated to nondegranulated GCs at baseline (0.75 ± 0.52) compared with the dry eye group (0.41 ± 0.27), the ratio became slightly higher in dry eye (2.04 ± 1.12 vs 1.99 ± 1.21 in control) after the ITN application. There was no significant difference between the IB or TB conjunctiva locations in terms of the effectiveness of the ITN application on conjunctival goblet cell secretory response.
• CONCLUSIONS: These preliminary results document that the Allergan ITN can stimulate degranulation of goblet cells in the conjunctiva, which is a promising new approach for the management of dry eye. (Am J Ophthalmol 2017;177:159–168.

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DRY EYE DISEASE (DED), WHICH HAS A COMPLEX pathophysiology and a multifactorial etiology related to an inadequate quantity or quality of tears, results in tear film instability, symptoms of discomfort, and visual disturbance that carries a risk for damage to the ocular surface epithelium.[1] Production, distribution, and clearance of tears from the ocular surface is tightly regulated by the integrated Lacrimal Functional Unit (LFU) that maintains tear stability in response to ocular surface demands via input from the sensory nerve endings in the cornea, conjunctiva, and eyelids.[2,3] The secretory components of the LFU are composed of the main and accessory lacrimal glands, conjunctival goblet cells, and meibomian glands. Dysfunction of LFU may lead to reduced tear secretion, delayed tear clearance, and reduced tear concentrations of factors produced by the secretory glands.[4–6] This can result from glandular dysfunction, reduced neural stimulation, or a combination of both.[7,8] Furthermore, the inadequately protected ocular surface in dry eye leads to ocular surface inflammation, which is known to be an important component of the pathophysiology of DED.[1,9–11] A vicious circle of inflammation on the ocular surface in dry eye may result in progressive loss of conjunctival goblet cells and their secreted mucus, which is correlated to chronic ocular inflammation and cell hyperosmolarity, and can further destabilize the tear film.[12,13]

Currently recommended management of DED includes artificial tears and anti-inflammatory agents.[11,13] Artificial tears contain polymers that lubricate the ocular surface, but they lack the numerous biological factors (antimicrobial, growth, and anti-inflammatory factors) found in natural tears that maintain ocular surface homeostasis.[8] Therefore, there is a need for a therapy that

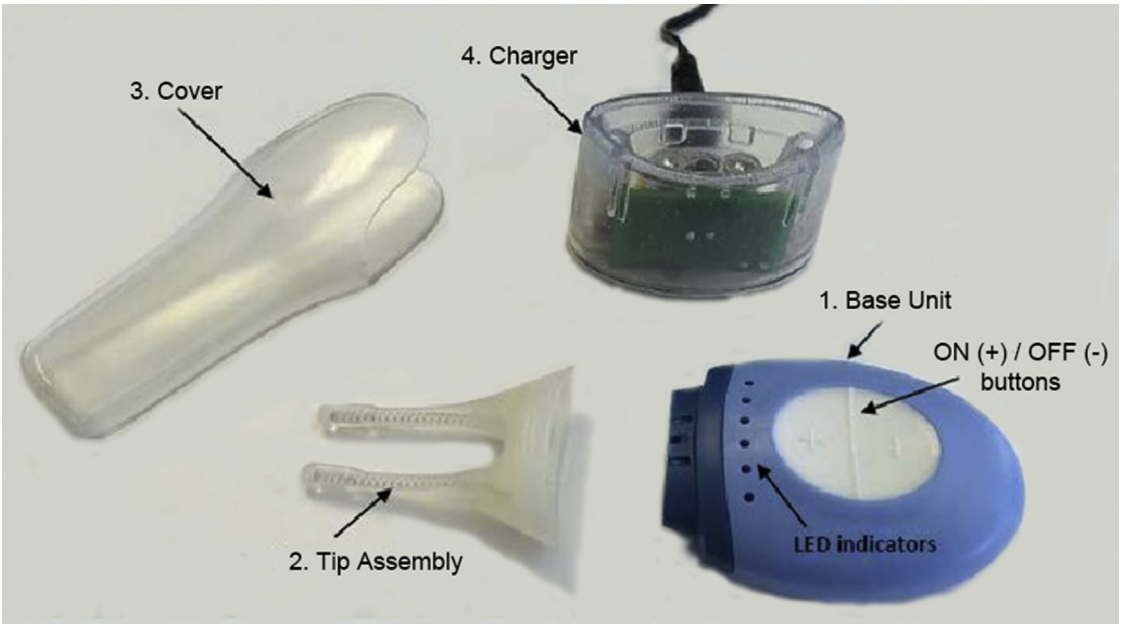

FIGURE 1. The intranasal neurostimulator used in this study to evaluate goblet cell degranulation and aqueous tear production consists of 4 distinct parts: (1) a reusable base unit, which produces the electrical stimulation waveform; (2) a disposable-tip assembly that inserts into the nasal cavity and stimulates the target intranasal tissue; (3) a reusable cover to protect the tip assembly; and (4) a charger, which recharges the battery inside the base unit.

stimulates secretion of natural tears from the secretory components of the lacrimal functional unit.

The Allergan Intranasal Tear Neurostimulator (ITN) represents a novel therapeutic approach to dry eye because it stimulates secretion by the tear glands, thus providing natural tears replete with biologically active constituents. The purpose of this study was to investigate the hypothesis that the Allergan ITN (Irvine, California, USA) might stimulate conjunctival goblet cell degranulation and mucus secretion, as well as stimulate lacrimal gland secretion to increase tear volume.

METHODS

• **SUBJECTS:** This study protocol was registered on ClinicalTrials.gov (#NCT02385292) and approved by the Baylor College of Medicine Institutional Review Board. It adhered to the tenets of the Declaration of Helsinki for clinical research and complied with the Health Insurance Portability and Accountability Act. Written informed consent was obtained from all participants after explanation of the purpose and possible consequences of the study. This is a prospective randomized controlled, crossover, multicenter clinical trial. Thirty eyes from 15 participants—10 dry eye patients and 5 subjects with no symptoms or signs of tear dysfunction—were involved in this study. All participants served as their own control.

TABLE 1. Inclusion Criteria for Dry Eye Patients

1	Be male and female subject of any race at least 22 years of age at visit 1
2	Baseline Ocular Surface Disease Index score of at least 23 with no more than 3 responses of "N/A" (not applicable)
3	Tear film breakup time ≤ 7 seconds in both eyes
4	In at least 1 eye, both corneal fluorescein staining ≥ 3 and conjunctival lissamine green staining ≥ 3 (National Eye Institute criteria)
5	In at least 1 eye, a Jones cotton swab nasal stimulation Schirmer test score at least 7 mm higher than the basal score in the same eye
6	Tear meniscus height ≤ 230 μm by optical coherence tomography
7	Normal lid/lash anatomy, blinking function, and closure as determined by the investigator
8	Stable treatment regimen for dry eye for 4 weeks prior to enrollment and during the course of the study

• **RANDOMIZATION:** The study design involved 3 visits consisting of 1 screening examination and 2 separate application days, during which they received 1 of the 2 applications (extranasal and intranasal), in randomized order, that were performed at least 1 week apart. A computer-generated randomization schedule was stratified by study site. Participants were not told which application was a control application and which was active.

FIGURE 2. To deliver the electrical stimulus from the device, subjects were instructed to place the tips of the Allergan Intranasal Tear Neurostimulator (ITN) into both nostrils simultaneously toward the top and front of the nose for the intranasal application (Left). Extranasal stimulation was performed as a control. In this case, subjects were instructed to place the tips of the ITN on the lower part of the nose skin (1 tip on each side) and apply the stimulus (Right).

• **ASSESSMENTS:** The same examiner (K.G.), who was masked to the application, performed all of the objective clinical tests. During the screening visit, all study participants underwent a detailed ophthalmologic examination including Ocular Surface Disease Index (OSDI) and dry eye symptom (Visual Analog Scale) questionnaires, corrected distance visual acuity, tear meniscus height (TMH) and area (TMA) measurements using anterior segment optical coherence tomography (ASOCT), biomicroscopy, tear film breakup time (TBUT) and corneal staining with fluorescein, conjunctival lissamine green staining, and Schirmer I test with and without cotton swab nasal stimulation.

During the application visits, ASOCT was performed before and immediately after the 3 minutes of intranasal or extranasal application. Impression cytology (IC) was taken from the temporal (TB) and inferior (IB) bulbar conjunctiva of the right eye for periodic acid–Schiff (PAS) staining and of the left eye for MUC5AC immunostaining of goblet cells at the end of each visit.

• **ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY–DEFINED TEAR MENISCUS PARAMETERS:** OCT measurement of the lower tear meniscus was performed as described in our previous studies.[14,15] All subjects underwent cross-sectional imaging of the lower tear meniscus prior to instilling drops or measuring any clinical parameters at baseline and before and after extranasal/intranasal applications using the RTVue-100 (Ver. 4.0.7.5; Optovue Inc, Fremont, California, USA) with a corneal adaptor. The long-CAM (13-mm, wide-field) lens adapter was used to take images. The subject was asked to fixate on a target but was allowed to blink freely throughout the duration of the measurement, and an image of the vertical cross section through the center of the lower tear meniscus was recorded within 2 seconds after a blink. The scan was repeated if artifact attributable to eye or eyelid movement was noted.

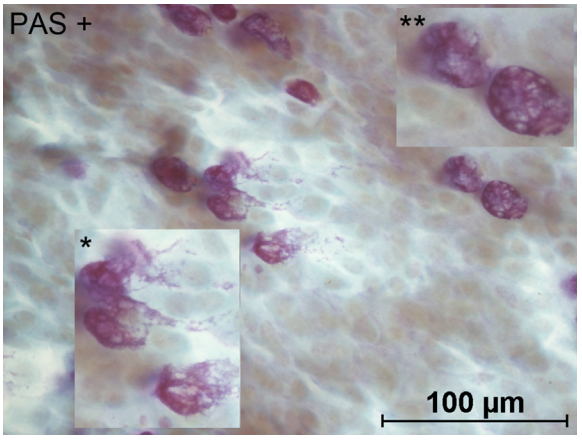

FIGURE 3. Image of periodic acid–Schiff (PAS)-stained impression cytology specimen showing degranulated (*) and nondegranulated (**) goblet cell types.

TMH and TMA were defined as the height and area of the triangular-shaped cross section between the lower eyelid margin and the cornea,[15] and were measured with RTVue-100 image analysis software.

The study eye was the eye with the lowest TMH prior to the first application or, if the TMH in both eyes were equal, the right eye was considered the study eye.

• **NEUROSTIMULATION:** Neurostimulation was performed using the Allergan ITN (Figure 1). There are 5 stimulation intensity levels and subjects may adjust the level by pressing the plus or minus button to obtain the best tingling "sneezy" sensation. The duration of stimulation can be controlled by how long they keep power on or the tips inserted.

Patients who meet all inclusion criteria (Table 1) were randomized to treatment sequence A (intranasal

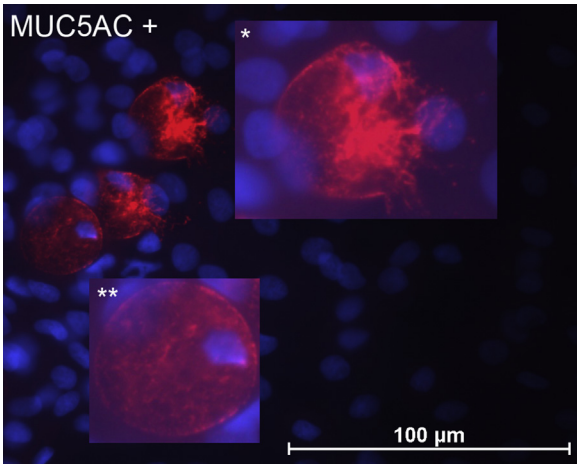

FIGURE 4. Image of mucin 5AC (MUC5AC)-stained impression cytology specimen showing degranulated (*) and nondegranulated (**) goblet cells.

TABLE 2. Demographic and Clinical Characteristics of Normal Subjects and Dry Eye Patients

Parameters	Normal Subjects (N = 5)	Dry Eye[a] (N = 10)	*P* Value
Age	42.2 ± 11.9	47.9 ± 8.6	>.05
Sex	3 F/2 M	9 F/1 M	>.05
OSDI	0	61.6 ± 22.1	*.002*
TBUT	9.7 ± 4.4	4.7 ± 1.3	*.014*
Corneal staining	0.2 ± 0.4	5.1 ± 2.0	*.002*
Conjunctival staining	2.2 ± 2.3	8.9 ± 3.3	*.005*
Schirmer I test	27.2 ± 8.3	10.6 ± 5.8	*.006*
Schirmer test[b]	ND	21.9 ±12.5	-

Italicized *P* values are considered statistically significant.

ND = not done; OSDI = Ocular Surface Disease Index; TBUT = tear breakup time with fluorescein.

[a]Dry eye group consisted of 5 subjects with Sjögren syndrome and 5 with non-Sjögren aqueous deficiency and meibomian gland dysfunction.

[b]Schirmer test performed after cotton swab nasal stimulation.

application at Visit 2 followed by extranasal application at Visit 3) or sequence B (extranasal application at Visit 2 followed by intranasal application at Visit 3).

Whereas patients were instructed to place the tips of the ITN into both nostrils simultaneously and advance toward the top and front of the nose (Figure 2, Left) for the intranasal application, they were instructed to place the tips of the ITN on the lower part of the nose (1 tip on each side, as shown in Figure 2, Right) for the extranasal application. In all applications, they were told to turn on the unit by holding down the plus (+) button for 3 seconds. Subjects were told they could cease stimulation by holding down the minus (-) button for 3 seconds on the stimulator or by withdrawing the tips from the nostrils.

• **IMPRESSION CYTOLOGY AND GOBLET CELL EVALUATION:** Eyeprim (Opia Technologies, Paris, France) was used to obtain sufficient cells from the right temporal and inferior bulbar conjunctiva. Membranes were fixed and stained by PAS reagent as previously described.[16]

IC samples were taken from the left temporal and inferior bulbar conjunctiva using Biopore membranes (EMD Millipore, Billerica, Massachusetts, USA) for MUC5AC immunofluorescence staining. The samples were fixed in cold methanol for 10 minutes, washed in 1% phosphate-buffered saline (PBS) twice for 5 minutes, and blocked with 1% bovine serum albumin (BSA) to reduce nonspecific staining for 1 hour. Rabbit MUC5AC antisera, prepared by NeoBiolab (Woburn, Massachusetts, USA) against reported MUC5AC sequences,[17] diluted 1:50 in 1% BSA was applied for 1 hour at room temperature. Membranes were washed with 1% PBS 3 times for 5 minutes each and then incubated with goat anti-rabbit IgG H & L (Alexa Fluor 594; Thermofisher, Atlanta, Georgia, USA) diluted 1:250 in PBS for 60 minutes at room temperature in a dark room. Membranes were washed with PBS 3 times for 5 minutes each and counterstained with 4′,6-diamidino-2-phenylindole DNA binding (Sigma Aldrich, St Louis, Missouri, USA) diluted 1:500 for 10 minutes. After washing with PBS 3 times for 5 minutes each, the membranes were carefully excised from the cylindrical holder, transferred to a glass slide, and covered with Gel Mount (Sigma-Aldrich; G0918) and a coverslip. Five images from each membrane were captured at ×20 magnification with a Nikon Eclipse epifluorescence microscope (Nikon, Garden City, New York, USA).

All images were processed with NIS Elements 4.20 version (Nikon). Goblet cells (GC) were counted in 5 representative images normalized per area and expressed as GC/mm^2. In addition to total goblet cell density (GCD), the ratio of degranulated to nondegranulated GCDs was measured as a marker of secretion. Nondegranulated GCs were typically well defined by their uniform size, intact cell borders, and intracellularly packaged mucins, while degranulated GCs were characterized by disrupted cell borders, scattered mucin granules, and mucin exocytosis, as shown in Figures 3 and 4. All stained cytology specimens were evaluated by an experienced person (K.G.) who was masked to application types and patients' clinical data. In pilot studies, we found tear volume returned to baseline within 4 hours after neurostimulation and the degranulated-to-nondegranulated GCD ratios returned to baseline within 1 week.

• **STATISTICAL ANALYSIS:** The data were analyzed using SPSS 20.0 for Mac (SPSS Inc, Chicago, Illinois, USA). Prior to statistical analysis, data distribution was checked for normality. Because of the sample size, nonparametric tests were preferred in the analysis. The Mann-Whitney *U* test was used to compare differences between 2

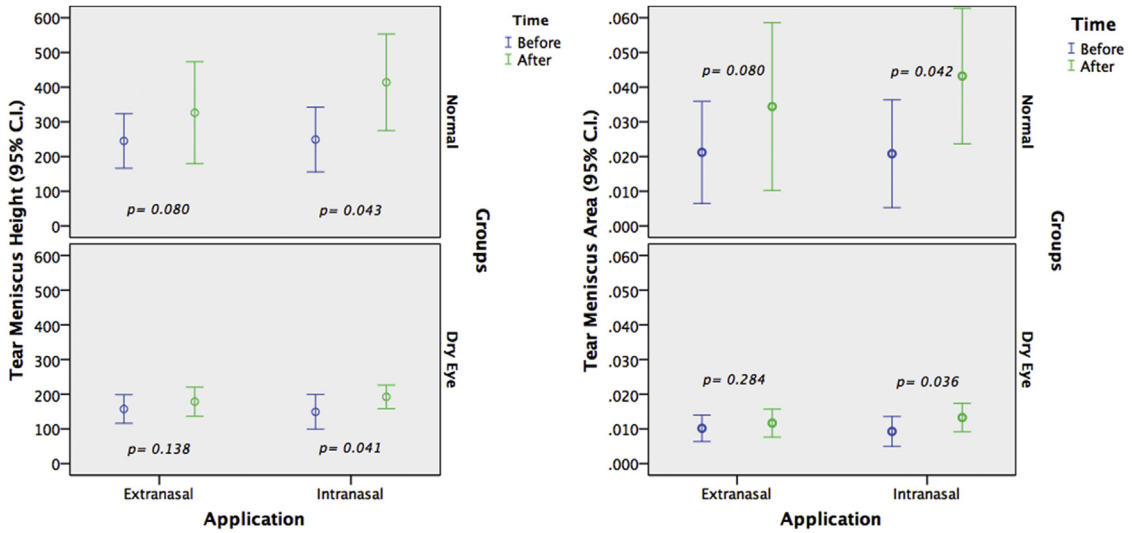

FIGURE 5. Means and 95% confidence intervals (CIs) for (Left) tear meniscus height and (Right) tear meniscus area values before and after extranasal and intranasal application in normal subjects and dry eye patients.

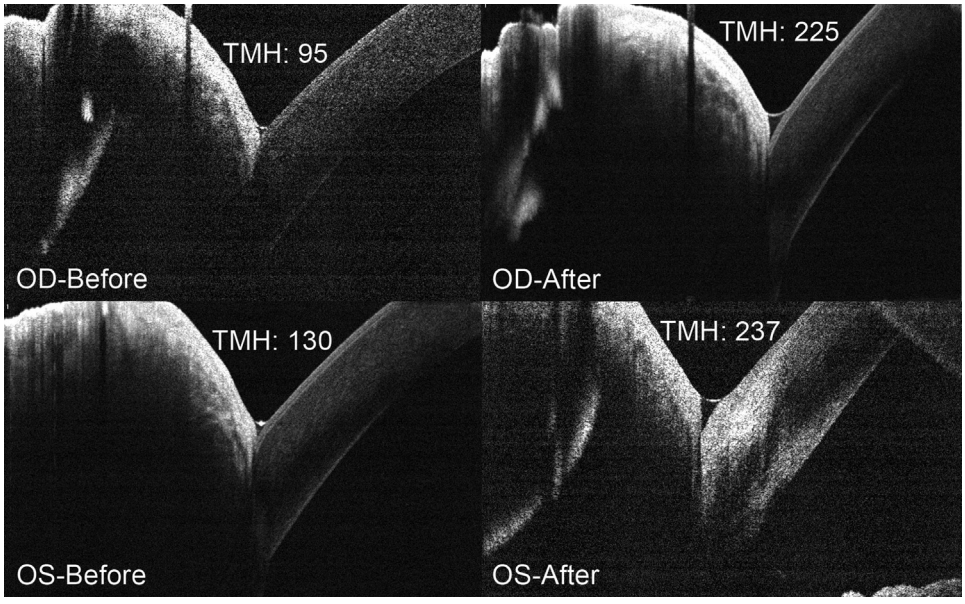

FIGURE 6. Optical coherence tomography images showing a change in tear meniscus height in a subject with Sjögren syndrome aqueous tear deficiency (Top: OD; Bottom: OS) before (Left) and after (Right) the intranasal application.

independent groups. The Wilcoxon signed rank test was used when comparing repeated measurements. A *P* value of less than .05 was considered as statistically significant.

RESULTS

THE BASELINE DEMOGRAPHIC AND CLINICAL CHARACTERistics of the study population are presented in Table 2.

A statistically significant increase in TMH and TMA after intranasal application was observed in both dry eye patients and normal subjects (Figure 5). An example of TMH change in 1 subject with Sjögren syndrome before and after the intranasal application is shown in Figure 6. Following the intranasal application, while mean TMH increased by 28.8% (from 149.50 μm to 192.60 μm) in dry eye patients, this increase was 66.3% (from 249 μm to 414 μm) in normal subjects.

GC degranulation was evaluated in cytology membranes stained with PAS reagent to detect glycoproteins

TABLE 3. Degranulated to Nondegranulated Goblet Cell Density Ratio in MUC5AC-Stained Impression Cytology Specimens of Dry Eye and Normal Subjects

Group	(A) Baseline	(B) Extranasal Application	(C) Intranasal Application	*P* Values		
				A-B	A-C	B-C
Dry eye (n = 20)	0.74 ± 0.62	0.57 ± 0.54	4.71 ± 4.48	.333	<.001	<.001
Normal subjects (n = 10)	0.75 ± 0.52	0.40 ± 0.22	1.99 ± 1.21	.082	.034	.001

Cells represent mean ± standard deviation in the temporal and inferior bulbar conjunctiva.

TABLE 4. Degranulated to Nondegranulated Goblet Cell Density Ratio in Periodic Acid–Schiff-Stained Impression Cytology Specimens of Dry Eye and Normal Subjects

Groups	(A) Baseline	(B) Extranasal Application	(C) Intranasal Application	*P* Values		
				A-B	A-C	B-C
Dry eye (n = 20)	0.73 ± 0.36	0.69 ± 0.39	2.02 ± 1.41	.606	.001	.001
Normal subjects (n = 10)	1.06 ± 0.72	1.02 ± 0.76	2.38 ± 0.84	.909	.007	.003

Cells represent mean ± standard deviation in the temporal and inferior bulbar conjunctiva.

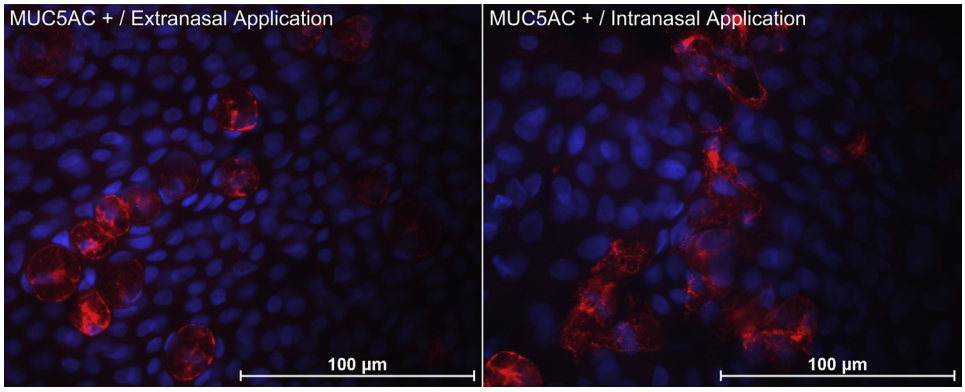

FIGURE 7. An example of mucin 5AC (MUC5AC)-stained impression cytology taken after extranasal (Left) and intranasal neurostimulation (Right) (×40 magnification). (Left) All goblet cells seem to be in a nondegranulated form with intact boundaries and packaged MUC5AC-stained mucous granules inside the cells. (Right) In contrast, most of the goblet cells showed a degranulation pattern with disruption of cellular morphology and exocytosis of MUC5AC-stained mucous granules and mucin outside the cells.

and with MUC5AC antisera to detect this GC-specific mucin. Immunostaining allowed high-resolution visualization of mucin-filled secretory vesicles in nondegranulated goblet cells. A significantly higher ratio of degranulated to nondegranulated GCDs was noted in MUC5AC-stained IC specimens from the dry eye group after intranasal stimulation (4.71 ± 4.48) compared to those taken at baseline (0.74 ± 0.62, $P < .001$) and after sham treatment (0.57 ± 0.54, $P < .001$). Similar results were obtained in PAS-stained IC specimens. Degranulated-to-nondegranulated GCD ratio values in dry eye and normal subjects stained with PAS and MUC5AC are given in Tables 3 and 4. An example of MUC5AC staining taken after extranasal and intranasal application is shown in Figure 7.

In MUC5AC-stained IC specimens from the dry eye group, even though degranulated-to-nondegranulated GCD ratio was higher in the unexposed conjunctiva (IB), there was no significant difference between the IB or TB conjunctiva locations in terms of the effectiveness of the ITN application on conjunctival GC secretory response (Figure 8).

Even though both the numbers (38.30 ± 35.11 vs 29.50 ± 24.85) and densities (79.96 ± 67.97 vs 70.58 ± 53.85) of degranulated GCs were higher in normal subjects than those in dry eye patients at baseline, the difference did

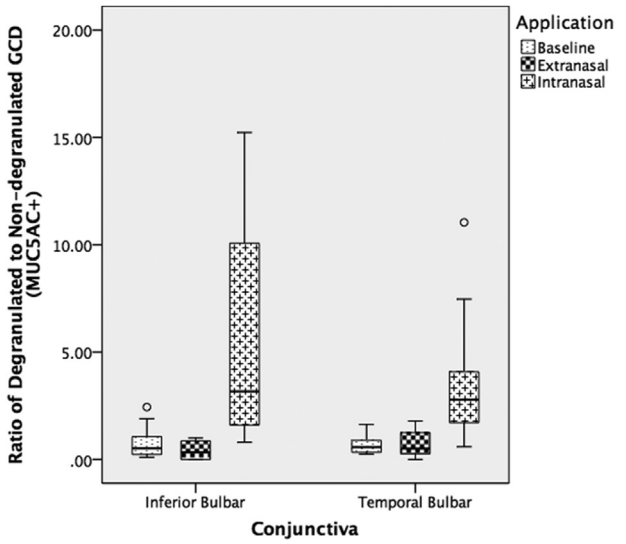

FIGURE 8. Ratio of degranulated to nondegranulated goblet cells in the inferior and temporal bulbar conjunctiva of dry eye patients in mucin 5AC (MUC5AC)-stained impression cytology membranes.

not reach a statistically significant level. After the ITN application, not only was the ratio of degranulated to nondegranulated GCs significantly increased, but the density of degranulated GCs was also increased, in both IB and TB conjunctiva locations of dry eye patients and normal subjects (Table 5).

When we analyzed the association of TMH change in the left eye with GCD ratio (degranulated/nondegranulated) in MUC5AC-stained IC specimens after the ITN application, a significant positive correlation was found in the normal subjects ($r = 0.712$, $P = .031$); the correlation did not reach statistical significance in dry eye patients ($P > .05$).

DISCUSSION

DRY EYE IS A COMMON OCULAR SURFACE DISEASE THAT IS characterized by tear film instability, symptoms of ocular discomfort, visual disturbance, and reduced quality of life.[16] Numerous studies have found evidence of ocular surface inflammation.[8,12,18] The cytokine interferon gamma (IFN-γ), which increased in aqueous-deficient dry eye, has been associated with GC loss and secretory dysfunction.[12,19,20] Conjunctival GC loss results in decreased mucus production and a poorly lubricated and irregular ocular surface.[21] Thus, preservation of GC number and function is vital for maintaining ocular surface homeostasis.

Although topical cyclosporine has been found to increase GC density,[22] it is unknown whether this or other agents stimulate mucin secretion by GCs. In this study, we hypothesized that the ITN, which was designed to trigger tear production by delivering a gentle electrical stimulation to the anterior ethmoidal branch of the trigeminal nerve in the upper nasal cavities, might stimulate conjunctival GC degranulation. Our data suggest that the application of Allergan ITN not only increases aqueous tear production, but also stimulates conjunctival GC mucin secretion, offering a promising new approach to treatment of dry eye.

Function of the lacrimal gland, which is an important part of the integrated LFU, decreases in aqueous deficiency owing to aging, autoimmune diseases (ie, Sjögren syndrome), neurotrophic conditions,[23] and graft-vs-host disease.[24] Previous studies have demonstrated that the lacrimal gland secretes in response to cholinergic (acetylcholine, vasoactive intestinal peptide) and α- and β-adrenergic neurotransmitters.[25–27] Tear secretion can also be induced by electrical stimulation of the afferent and efferent nerves proximal to the lacrimal gland.[28,29] One study that investigated the response pathways and electrical parameters to maximize tear secretion documented that electrical stimulation of the lacrimal gland engages primarily the efferent cholinergic pathway to enhance tear secretion.[30]

Increasing tear production using electrical stimulus of the lacrimal gland was first attempted in animal models.[28,30] In one study, Kossler and associates implanted a neurostimulator adjacent to the right lacrimal nerve.[28] After 2 minutes of lacrimal nerve stimulation (LNS) (100 μs, 1.6 mA, 20 Hz, 5–8 V), the authors measured tear volume using ultra-high-resolution OCT (UHR-OCT). The UHR-OCT revealed a 441% average increase in tear production after LNS. Furthermore, histopathologic examination of the lacrimal glands showed no tissue damage from chronic neurostimulation.[28] In another study, Brinton and associates implanted bipolar platinum foil electrodes beneath the inferior lacrimal gland and placed a monopolar electrode near the afferent ethmoid nerve of rabbits.[30] A 4.5-mm (125%) increase in the Schirmer test was noted after lacrimal gland stimulation with 3-mA, 500-μs pulses at 70 Hz. The authors also concluded that modulating the stimulation waveform (1 second on, 1 second off) generated the strongest response in the chronically implanted animals, and ethmoid nerve stimulation produced a tear secretory response similar to lacrimal gland stimulation via a reflex pathway.[30]

Based on the success achieved with lacrimal gland or anterior ethmoidal nerve stimulation, a noninvasive intranasal lacrimal neurostimulator was developed and tested.[31] This ITN is a hand-held device designed to trigger natural tear production by delivering gentle electrical currents to the ethmoidal nerve, a sensory subunit of the ophthalmic branch of the trigeminal nerve that innervates the nasal cavities, and leads to an increase in activity in the superior salivatory nucleus, which supplies cholinergic efferents that stimulate natural lacrimation.[31] In addition to sensory

TABLE 5. Degranulated Goblet Cell Densities in MUC5AC-Stained Impression Cytology Specimens of Dry Eye and Normal Subjects

Groups	Conjunctiva	Baseline	Extranasal Application	Intranasal Application
Dry eye	IB (n = 10)	75.11 ± 53.57	44.39 ± 40.79	175.25 ± 112.22
	TB (n = 10)	67.79 ± 62.11	52.28 ±45.62	128.52 ± 86.01
	Combined (n = 20)	71.04 ± 56.91	48.77 ± 42.46	149.29 ± 98.35
Normal subjects	IB (n = 5)	99.06 ± 96.28	67.23 ± 29.45	124.87 ± 39.56
	TB (n = 5)	69.73 ± 52.78	20.66 ± 13.69	85.76 ± 84.32
	Combined (n = 10)	82.77 ± 71.47	41.36 ± 31.96	103.15 ± 67.58

IB = inferior bulbar; TB = temporal bulbar.

neural stimulation from the ocular surface, it is recognized that sensory stimulation of the nasal mucosa plays a crucial role in stimulating homeostatic aqueous tear production.[32] Gupta and associates investigated the effect of nasal mucosal anesthesia on aqueous tear production and found a 34% decrease in Schirmer I scores from baseline after nasal anesthesia.[32]

A preliminary report of the efficacy and safety of nasal electrical stimulation in patients with dry eye was recently reported. In that study, 40 patients with mild to severe dry eye were instructed to use the nasal stimulator 4 times daily for almost 180 days.[31] The authors found that mean Schirmer scores were significantly higher than those of the unstimulated scores at all visits, and both corneal and conjunctival staining and symptom scores were significantly reduced from baseline at day 180.[31] In the same study, patient diary data revealed an improvement in ocular comfort lasting for almost 3 hours after the intranasal application.[31]

In the current study, we assessed the acute effects of ITN on tear dynamics and GC secretion. For this purpose, all study participants (5 normal subjects and 10 dry eye patients) received 2 separate randomized, masked intranasal or sham extranasal ITN treatments. All participants were instructed to use the Allergan ITN only once for 3 minutes and the level of stimulation could be selected by the patient. TMH and degranulated-to-nondegranulated GC ratio in conjunctival IC were assessed at baseline and post treatment. There was a significant increase in TMH after intranasal compared to sham extranasal treatment in both normal and dry eye groups ($P = .04$ for both). An increase of TMH following the ITN application was 28.8% in dry eye patients and 66.3% in normal subjects. Interestingly, a very slight increase in TMH values was observed after the sham/extranasal application. This finding might be owing to stimulation of the anterior ethmoidal nerve endings that innervate skin on the nose during the extranasal application. Similar to the long-term safety and efficacy study, we found increased tear production following acute ITN.[31] These studies clearly document that stimulating sensory nerves inside the nasal cavity can increase production of natural tears in dry eye patients. However, some questions remain regarding how long the increased aqueous or mucus tear volume lasts after a single application and how many times per day neurostimulation would need to be performed to maintain the effect.

In addition to evaluating lacrimal secretion, one of the strengths of our study was the ability to investigate GC morphology and mucin secretion after ITN. GC degranulation was evaluated by impression cytology taken before and after ITN. One set of membranes was stained by PAS to detected secreted glycoproteins, while immunostaining for MUC5AC, the predominant mucin produced by conjunctival goblet cells that stabilizes the tear film, was performed on the other set. Instead of measuring the density of GCs in cytology specimens, considerable attention was given to morphologic changes and integrity of GCs. Specifically, the location of mucin (ie, packaged inside the cell or not) and its appearance (ie, expulsive appearance of mucins) was documented to discriminate degranulated and nondegranulated GCs, particularly in MUC5AC-stained specimens. Consequently, a significantly higher ratio of degranulated to nondegranulated GCs was noted in MUC5AC-stained cytology specimens from the dry eye group after intranasal stimulation (4.71 ± 4.48) compared to those taken at baseline (0.74 ± 0.62, $P < .001$) and after sham treatment (0.57 ± 0.54, $P < .001$). Similar findings were observed in the PAS-stained cytology specimens.

Remarkably, there were some clusters composed of only degranulated GCs both in PAS- and MUC5AC-stained specimens after intranasal application, whereas none of these clusters was observed in the cytology specimens taken at baseline and after extranasal application. Furthermore, not only was an increased degranulated-to-nondegranulated GC ratio noted after intranasal application, but also a significant increase in the mean degranulated GC density was observed after ITN. There was no significant difference between the IB and TB conjunctiva locations in terms of the effectiveness of the ITN application on conjunctival GC secretory response. Another important finding of the current study was the existence of significant correlation between TMH change and degranulated-to-nondegranulated GC ratio in

MUC5AC-stained IC membranes of normal subjects; however, no significant correlation was found in dry eye patients. Confirmation of GC secretion by measuring of MUC5AC in tears would have been ideal; however, accurate measurement of MUC5AC, which is a large-molecular-weight glycoprotein, in tears is a difficult because the mucin molecule sticks to pipette tips and plasticware. Additionally, induction of reflex aqueous secretion can dilute tear MUC5AC concentrations, which could actually appear to decrease following neurostimulation. This was observed for the abundant tear protein lactoferrin in pilot studies (data not shown).

These findings indicate that ITN can trigger conjunctival GC mucin secretion, which may be a unique feature of this therapy compared to other currently available treatments. In other words, ITN appears to offer a promising new approach to treatment of dry eye because it stimulates aqueous tear production by the lacrimal glands as well as mucin secretion by conjunctival goblet cells.

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