A New Matrix Therapy Agent for Faster Corneal Healing and Less Ocular Discomfort Following Epi-off Accelerated Corneal Cross-linking in Progressive Keratoconus

Koray Gumus, MD, FEBOphth; Marta Gomes Guerra, MD, MSc; Sara Homem de Melo Marques, MD, MSc; Sarper Karaküçük, MD, AME; Denis Barritault

ABSTRACT

PURPOSE: To investigate the hypothesis that a new matrix therapy agent (ReGeneraTing Agent, [RGTA]) would speed up the corneal reepithelialization, improve stromal healing, and reduce ocular symptoms after epi-off corneal cross-linking (CXL).

METHODS: Sixty eyes of 60 patients with progressive keratoconus were enrolled in the study. Epi-off accelerated CXL was performed in all patients. Sixty eyes were randomized into two groups according to use of RGTA eye drops prior to contact lens fitting at the end. Identical medical agents were started postoperatively for the two groups. All participants were monitored on 3 consecutive days after the CXL. Ocular pain, burning, stinging, tearing, photophobia, conjunctival hyperemia, and corneal healing status were evaluated.

RESULTS: By day 2, 25 eyes (83.3%) with RGTA revealed complete healing compared to 4 eyes (13.3%) that revealed complete healing in the control group ($P < .001$). All eyes had complete corneal epithelial defect closure by day 3 in both groups. Ocular pain scores were lower in the RGTA group on days 0, 1, and 2 (all $P < .05$). Burning scores were lower on days 1 and 2; stinging scores on days 2 and 3; tearing scores on days 2 and 3; and photophobia on days 1 and 2 ($P < .05$) in the RGTA group compared to the control group.

CONCLUSIONS: RGTA ophthalmic solution facilitates corneal healing by reconstructing the extracellular matrix in the wound area, leading to an earlier relief of symptoms for patients.

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Corneal cross-linking (CXL) has been used widely throughout the world since its safety and successful impact in halting the progression of keratoconus was proved by numerous studies.[1-4] The procedure can be done by either removing the epithelium or keeping the epithelium intact during the treatment.[3,5,6] Although some authors have claimed that these two techniques have equivalent effects on clinical outcomes, most of the studies to date have shown that the epithelium needs to be removed to optimize results after CXL because the epithelium blocks absorption of riboflavin.[7-9]

Although the epi-off technique was found to improve the efficiency of CXL, removing the epithelium may have some disadvantages, including postoperative ocular discomfort, an increased risk of corneal infections, delayed epithelialization, and haze.[10]

From the Ocular Surface & Contact Lens Division, Department of Ophthalmology, Erciyes University School of Medicine, Kayseri, Turkey (KG); the Department of Ophthalmology, Centro Hospitalar Tondela-Viseu, Viseu, Portugal (MGG, SHMM); the Department of Ophthalmology, Anadolu Medical Center in affiliation with Johns Hopkins Medicine, Gebze, Kocaeli, Turkey (SK); and Université Paris-Est Créteil, Laboratoire CRRET and OTR3, Paris, France (DB).

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Correspondence: Koray Gumus, MD, FEBOphth, Department of Ophthalmology, Erciyes University School of Medicine, Kayseri, Turkey, 38039. E-mail: drkorayg@hotmail.com

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To accelerate the healing process of the corneal epithelium after the epi-off technique, new pharmaceutical agents have been developed. A new type of matrix therapy agent (ReGeneraTing Agent [RGTA]) has gained popularity in challenging cases. Early studies have provided encouraging results, accelerating the healing of chronic skin ulcers of diabetic or vascular origin.[11,12] These agents consist of large (80,000 KD) substituted polysaccharide (Alpha 1-6 polycarboxymethylsulphate),[13] which replace destroyed heparan-sulfate molecules, both as an element of the extracellular scaffold bridging matrix proteins and as a depot and protector of growth factors and other communication peptides, thereby restoring a cellular microenvironment reminder of the initial healthy tissue.[14] RGTA (OTR4120 Cacicol; Laboratoires Théa, Clermont-Ferrand, France) has also been used in ophthalmology with encouraging results in the treatment of corneal ulcers and dystrophies of various etiologies.[15-17]

We tested the hypothesis that RGTA combined with the standard treatment would speed up the corneal healing and reduce ocular symptoms after the epi-off CXL compared to the standard treatment alone.

PATIENTS AND METHODS

Patients

The study was conducted according to the tenets of the Declaration of Helsinki. Informed consent was obtained from each patient after a full explanation of the procedure. Sixty eyes of 60 patients with progressive keratoconus were enrolled in this prospective, randomized, single-masked study on RGTA combined with standard treatment versus standard treatment alone. Exclusion criteria were age younger than 18 years, a history of any previous ocular surgery, a history of ocular trauma, and any ocular surface disease (eg, active ocular inflammation/infection, corneal dystrophies, and dry eye and corneal dystrophies) that might affect the results.

Accelerated CXL With Riboflavin/UVA

Accelerated CXL with riboflavin and ultraviolet-A (UVA) were performed by the same experienced surgeon (KG) in all patients. Topical anesthesia was achieved with 0.5% proparacaine hydrochloride eye drops preoperatively. The central 8 to 9 mm of the corneal epithelium were removed using a modified technique, which was the combination of alcohol delamination and mechanical debridement.[18] In this technique, only the tip of a triangle sponge was soaked in 20% ethanol and allowed to touch the corneal surface for 20 seconds meticulously. Then the ocular surface was rinsed thoroughly with 30 mL of balanced salt solution. Finally, the epithelium was loosened and peeled by the movement of the dry cellulose triangle sponge and debrided using a blunt spatula. Eyes were kept open by an eyelid speculum during riboflavin instillation every 2 minutes for 30 minutes (Collagex, isotonic 0.1%; Lightmed USA Inc., San Clemente, CA) The UV lamp (UV-X 2000 illumination system; IROC AG, Zurich, Switzerland) was then focused on the apex of the cornea at a distance of 5 cm for a total of 10 minutes, providing a radiant energy of 9 mW/cm^2. During UVA administration, riboflavin drops were applied to the cornea every 2 minutes. Pachymetry values (at five different locations) were continuously monitored to ensure that none of them dropped below 400 µm. At the end of the CXL, 60 eyes were randomized into two groups according to the use of an RGTA eye drop (Cacicol20; Laboratoires Théa) prior to contact lens fitting. Thirty eyes received one drop of RGTA (test group) combined with standard treatment, whereas the other 30 eyes (control group) only received the standard treatment without RGTA. RGTA is currently approved in Turkey for the management of chronic corneal wound healing, such as in persistent epithelial defects with associated pain.

Postoperatively, all patients were asked to use the same medical agents in the same frequency, consisting of preservative-free artificial tears (polyvinyl alcohol, Allergan, Inc., Irvine, CA) six times per day for 1 month and topical antibiotic (0.5% moxifloxacin, Alcon Laboratories, Inc., Fort Worth, TX) three times per day for 7 days. The same brand of therapeutic bandage contact lens (Night & Day; Ciba Vision, Alcon Laboratories, Inc.) was used at the end of the procedure. All patients were permitted to take the same oral analgesic medication (dexketoprofen 25 mg) as needed, with a maximum of 75 mg daily. Following complete corneal healing, topical steroid (0.1 % dexamethasone) was started four times per day after the last visit (day 3).

Evaluation of Ocular Symptoms and Conjunctival Hyperemia

All participants were monitored for 3 consecutive days after the CXL procedure. Ocular symptoms including pain (semi-qualitative Visual Analogue Scale graded on a scale of 0 to 10), burning, stinging, tearing, and photophobia (graded on a scale of 0 to 3) were noted at each visit. If the patient had used an oral analgesic, the number of pills was noted for each day. All patients were instructed to note the highest score they felt throughout the day. Conjunctival hyperemia was scored using a standardized photographic scale derived from McMonnies grading (1 to 6).

Evaluation of Corneal Epithelial Defect

On days 2 and 3, contact lenses were removed. After using sterile fluorescein strips, corneal epithelial defect healing was evaluated by the same experienced surgeon

(KG) under the cobalt blue light of the same slit-lamp biomicroscope in combination with a digital camera (SL 990 Elite HR; CSO Ophthalmic Instrument Company, Auckland, New Zealand). Anterior segment images were taken in all cases at the same magnification (×6) under the same room illumination. The photographs were saved as a JPEG file format (Phoenix software, version 2.6; Phoenix Software International, Inc., El Segundo, CA).

If there was incomplete healing, the size of the corneal epithelial defect was measured by using two different methods. In the conventional technique, the size of the corneal epithelial defect was measured in two linear dimensions, the longest linear diameter and the largest one perpendicular to it with the help of a surgical caliper. The estimated area of the corneal epithelial defect was calculated by multiplying the two measured perpendicular linear dimensions.[17] In the image analysis technique, the size of the stained corneal epithelial defect area was determined by point-to-point using image analysis software (ImageJ; version 1.47; Wayne Rasband Research Service Branch, National Institutes of Mental Health, Bethesda, MD). At each visit, corneal epithelial defect size was calculated as a proportion of the total corneal area by slit-lamp photography. Area measurements were made by two independent observers (MGG and SHMM) with no access to patient data or clinical stage. Each observer performed every calculation twice.

Interobserver and Intraobserver Variability

Intraclass correlation coefficients (ICCs) analysis was performed for the calculation of digital corneal epithelial defect/cornea ratio to see its reliability and repeatability in the same 31 corneal images with stained corneal epithelial defect. To evaluate interobserver and intraobserver variability, measurements were initially made twice by the first examiner (MGG) on different days. Next, a second examiner (SHMM) who had no knowledge of the first examiner's measurements obtained results using the same technique and images. Finally, the mean of the repeated measurements taken by the first examiner was used for statistical analysis.

Statistical Analysis

The data were analyzed using SPSS 20.0 for Macintosh software (SPSS, Inc., Chicago, IL).

Prior to statistical analysis, data distribution was checked for normality. An independent-sample *t* test was conducted to compare the continuous variables between the two groups. Categorical variables were compared using the Fisher's exact test. A *P* value of less than .05 was considered statistically significant. Scatterplots illustrating the intraoperative and interobserver correlations are shown in **Figure 1**.

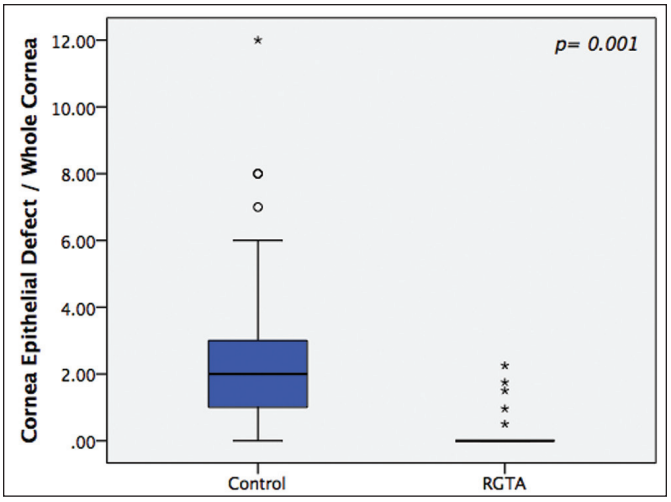

Figure 1. This boxplot reveals that topical ReGeneraTing Agent (RGTA) has a significant effect on the healing of corneal epithelial defect on day 2.

RESULTS

Mean age and gender distribution were comparable in the two groups (*P* = .775 and .422, respectively). The control group contained 30 patients with a mean age of 29 years (range: 18 to 48 years) and a male/female ratio of 17 of 13. The RGTA group contained 30 patients with a mean age of 29.67 years (range: 18 to 48 years) and a male/female ratio of 21 of 9.

The calculation of digital ratio (corneal epithelial defect size/whole corneal size) using the ImageJ software revealed excellent ICCs for intraobserver (Cronbach's alpha coefficient: 0.999, 95% confidence interval: 0.999 to 1.000, $P < .001$) and interobserver reliability (Cronbach's alpha coefficient: 0.990, 95% confidence interval: 0.935 to 0.997, $P < .001$) in the reliability analysis. Intraobserver and interobserver correlation plots are provided in **Figure A** (available in the online version of this article).

There was no significant difference between the study groups in terms of corneal topographical parameters including simulated, average, and maximum keratometry values (Sim K1, Sim K2, K_{avg}, and K_{max}, respectively), Baiocchi Calossi Versaci index (BCV), and thinnest corneal thickness (**Table A**, available in the online version of this article).

On day 2, 25 eyes (83.3%) treated with RGTA had complete corneal epithelial defect healing compared to 4 eyes (13.3%) in the control group ($P < .001$). The corneal epithelial defect area on day 2 was 0.23 ± 0.58 mm^2 (range: 0 to 2.25 mm^2) in the RGTA group and 0.17 ± 0.46 mm^2 (range: 0 to 12 mm^2) in the control group ($P < .001$). The ratio of CED to whole cornea on day 2 was 0.17 ± 0.46 mm^2 (range: 0 to 1.75 mm^2) in the RGTA group and 2.97 ± 4.15 mm^2 (range: 0 to 19.51 mm^2) in the control group ($P = .001$) (**Figure 1**). Cor-

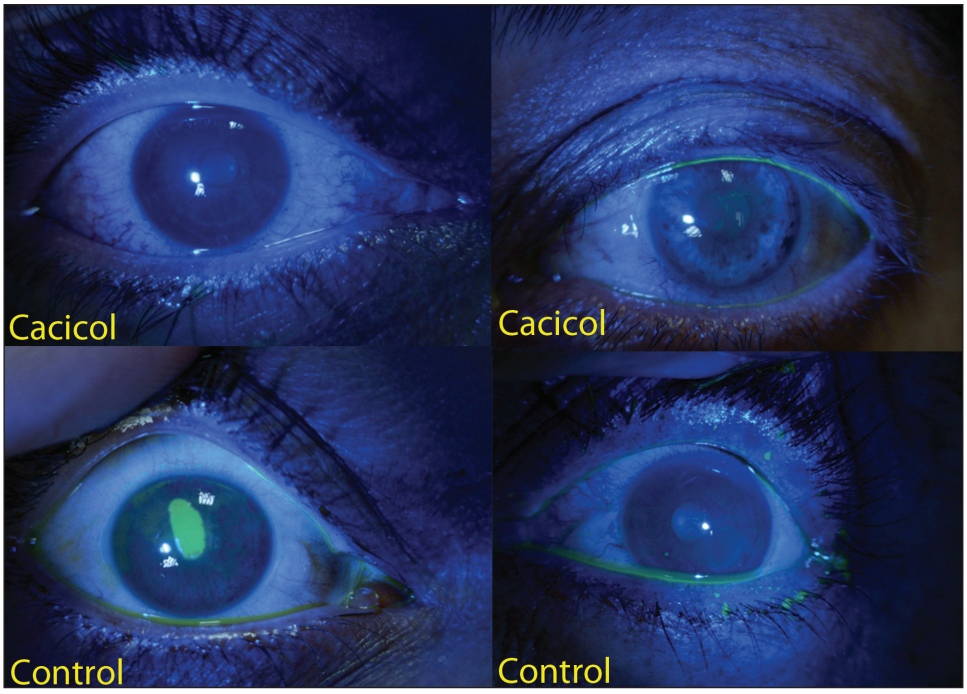

Figure 2. Different corneal healing patterns in ReGeneraTing Agent (RGTA) (OTR4120 Cacicol; Laboratoires Théa, Clermont-Ferrand, France) and control groups on day 2.

neal images of two patients on day 2 who used RGTA and who did not are shown in **Figure 2**. All epithelial defects were completely closed in all study groups by 72 hours. Some patients still had superficial punctate staining, mainly in the control group.

A comparison of two groups in terms of ocular symptoms (ocular pain, burning, stinging, tearing, and photophobia) and conjunctival hyperemia scores on 3 consecutive days after epi-off CXL is shown in **Figure 3**. Ocular pain scores were found to be lower in the RGTA group on days 0, 1, and 2 (P = .031, .025, and .001, respectively). When compared to controls, the RGTA group showed statistically significant lower burning on days 1 and 2, stinging on days 2 and 3, tearing on days 2 and 3, and photophobia on days 1 and 2 ($P < .05$). Conjunctival hyperemia scores were lower in the RGTA group on days 1 and 2 (P = .001). All subjective scores and the number of oral analgesics used daily were combined to obtain the composite scores for each participant for further statistical analysis (**Figure 4**).

The number of oral analgesics taken after the epi-off CXL was found to be significantly lower on days 0, 1, and 2 in the RGTA group (P = .007, .001, and .012, respectively). On further analysis, it was concluded that the number of oral analgesics taken after the procedure in all patients seemed to be correlated with ocular pain scores on days 0, 1, and 2 (r = 0.519 and $P < .001$, r = 0.535 and $P < .001$, and r = 0.358 and P = .005, respectively).

No adverse effects or ocular complications related to CXL or any topical medications (including RGTA) were reported during the follow-up period.

DISCUSSION

This study investigated the efficacy and safety of topical RGTA ophthalmic solution, which is a biodegradable nanopolymer engineered to mimic glycosaminoglycans for providing faster corneal epithelialization and stromal healing with fewer ocular symptoms after epi-off CXL in progressive keratoconus. Our results are compatible with the hypothesis that topical RGTA ophthalmic solution, used only once at the end of the epi-off technique prior to bandage contact lens fitting, accelerates corneal healing and decreases ocular discomfort such as ocular pain during the follow-up period.

Corneal healing is a complex process involving cellular interaction and various molecules such as proteases, growth factors, and epithelial and stromal cytokines.[17,20] The signaling proteins, cytokines, interleukins, chemokines, and extracellular matrix play a crucial role in the maintenance of cellular communication and homeostasis.[21] Glycosaminoglycans contribute to the regulation of this cellular communication[17]; in particular, heparan sulfates anchor and protect various growth factors and provide structure to the extracellular matrix by linking various structural proteins such as collagen, elastin, and fibronectin.[17,22] Heparan sulfate mimetic polymers, also named RGTAs, were engineered to replace the heparan sulfates bound to matrix proteins and growth factors in damaged cornea. RGTA is expected to bind to "heparan binding sites" on matrix proteins that become available as glycosaminoglycans are degraded by glycanases activated during the inflammation. It is

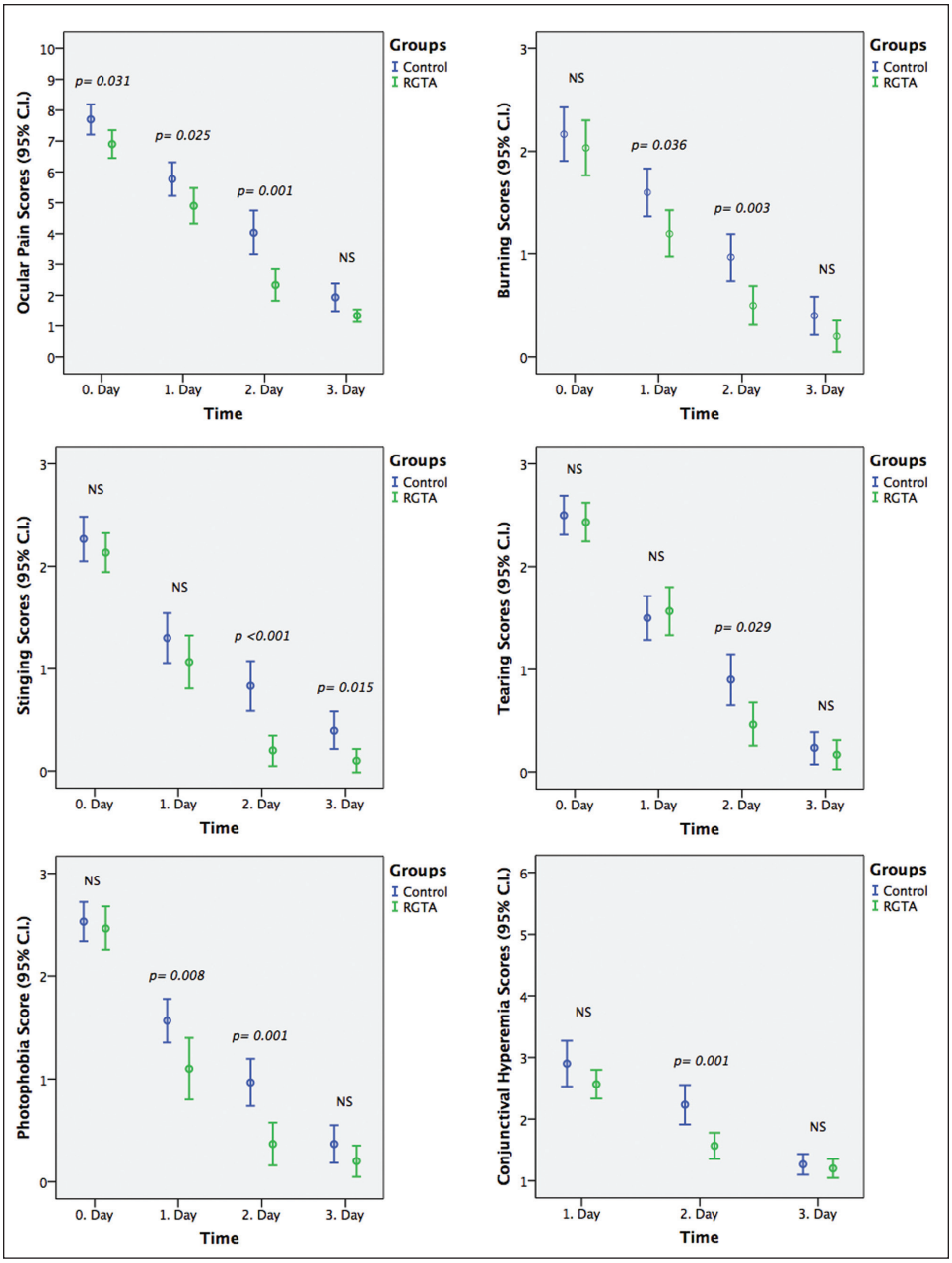

Figure 3. Error plots reveal ocular symptoms and conjunctival hyperemia scores in the ReGeneraTing Agent (RGTA) and control groups. CI = confidence interval

large enough to bridge matrix proteins and reconstitute a scaffold to which heparan-binding communication peptides (most growth factors, cytokines, and chemokines) synthesized by neighboring cells can bind in a space organization. RGTAs are not degraded by glycanases, hence the communication peptides bound to the new scaffold will be protected (by steric hindrance) from protease degradation. Similarly, matrix proteins will also be protected. Hence, RGTA will recreate a cellular microenvironment and a niche where cells respond properly to the cascade of signals needed for tissue regeneration, resulting in improved repair and wound healing.[11,12,17,21] This restored microenvironment will favor cell homing and this applies to both corneal and epithelial cells. However, in the case of CXL, it is unlikely that cross-linked stromal proteins will be homing keratocytes migrating from other areas of the stroma because riboflavin is a small molecule that links too tightly and covalently to proteins. Hence, in this case we expect only epithelial cell healing and scar improvement of the stromal matrix with reduced inflammation. RGTAs have been shown to modulate collagen synthesis in several cell culture and tissue explant models, as well as affect the enzymatic activities of various proteases involved in extracellular matrix remodeling.[12,23,24] Briefly,

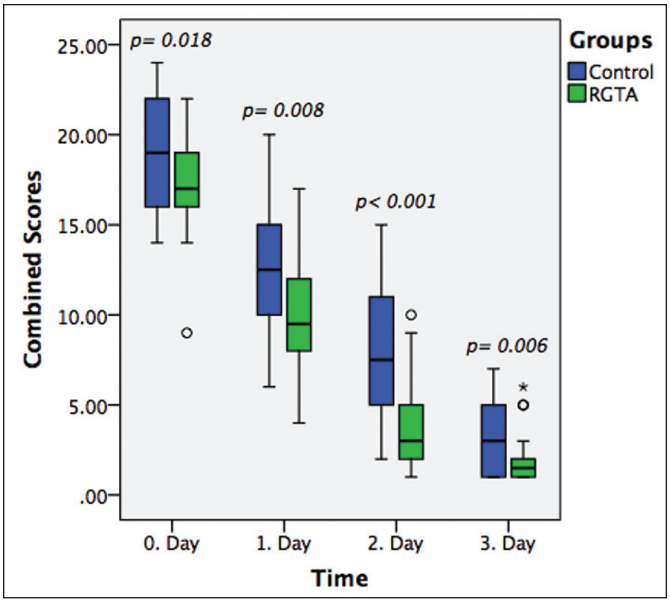

Figure 4. This boxplot reveals combined symptom scores in the ReGeneraTing Agent (RGTA) and control groups during consecutive visits on the day and after the epi-off accelerated corneal cross-linking.

RGTA facilitates the healing of injured corneas via a reduction of proteolytic, oxidative, and nitrosative damage.[25]

In the literature, in vivo and in vitro experimental models concluded that various RGTAs significantly improved the speed and quality of wound repair in different tissues such as skin and also decreased wound-related pain.[11,12] One recent study in a rabbit corneal model concluded that RGTA-based matrix therapy not only provided reepithelialization, but also reduced clinical signs of inflammation and improved histological patterns such as edema, fibrosis, neovascularization, and inflammation.[26]

There is currently limited data on the effect of RGTAs on corneal wound healing with different etiologies. Chebbi et al. evaluated the tolerance and safety of a new ophthalmic solution based on RGTA technology in corneal ulcers and severe chronic dystrophies.[15] In their study, RGTA ophthalmic solution was instilled as a single drop during each weekly visit over 1 month. Ocular pain scores were recorded using the Visual Analogue Scale pain scale ranging from 0 to 100. The group concluded that this new RGTA ophthalmic solution was found to be well tolerated with no side effects and provided favorable corneal healing in almost all corneal ulcers.[15]

Neurotrophic corneal ulcers are one of the most challenging corneal wounds to manage due to the high failure rate of current treatment modalities. Topical treatment with RGTA eye drops for corneal neurotrophic ulcers could be considered as a potential alternative treatment regimen. Results in the current literature have been encouraging.[16,17] In one uncontrolled, prospective, single-center clinical trial on 11 eyes with severe corneal neurotrophic ulcers, patients were treated with RGTA eye drops at a dosage of one drop in the morning on alternate days.[17] Eight eyes revealed complete corneal healing after a mean period of 8.7 weeks; three eyes required further surgical treatment because of treatment failure with RGTA eye drops.[17] In one case report by De Monchy et al.,[16] the authors used a new RGTA eye drop (Cacicol) for neurotrophic ulcers, with a dosage of two eye drops per week for 6 weeks. Complete healing was obtained in less than 3 weeks, with no side effects.

RGTA eye drops could also be considered in procedures requiring corneal epithelial removal such as epi-off CXL or photorefractive keratectomy. In the literature, there is only one published study about this indication.[27] Kymionis et al. evaluated the effect of an RGTA (Cacicol-poly-carboxymethyl glucose sulfate) eye drop on corneal healing and ocular pain in 18 patients after CXL.[27] In their study, one eye of each patient randomly received Cacicol once a day and the fellow eye received artificial tears. Eyes were evaluated in terms of corneal healing every day after CXL. The authors documented significantly lower mean epithelial defect size in the study group (RGTA group) on days 1 and 2. On day 3, 61.1% of study eyes were fully reepithelialized compared with 11.1% of control eyes. However, no significant effect in subjective ocular pain was observed between the groups. These results are slightly different when compared to our results. First, most of our cases (83.3%) in the RGTA group revealed complete corneal healing on day 2, which was 1 day earlier. Second, ocular pain scores were found to be significantly lower in the RGTA group on days 0, 1, and 2 in our study. Additionally, other ocular discomfort scores, including burning, stinging, tearing, and photophobia, appeared to be less in the RGTA group, particularly on day 2.

These differences might be explained by the differences in methodology. For instance, one important possible reason is that skipping corneal healing evaluation on day 1, as was done in our study, might have enhanced the efficacy of RGTA eye drops. Dosing regimen differences may have also affected results. In our study, RGTA was administered once directly in contact with the corneal stroma (target site) and just before inserting the contact lens, which may have protected RGTA interactions with the collagen fibers of the stroma. In Kymionis et al.'s study, RGTA was administered on the surface of the contact lens once every day, which was slightly overdosed in comparison with the recommended dose (one drop every day).[27] A brief explanation of extracellular matrix remodeling may help us to understand the possible ad-

verse effect of overuse on wound healing. In a damaged inflamed tissue, extracellular matrix components are actively destroyed, endocytosed, and degraded in the lysosomes of the cell, and newly synthetized matrix elements are secreted outside the cell to form a new extracellular matrix.[28] Once RGTA is applied, it occupies available sites, which are then saturated, and participates in the reconstruction of the extracellular matrix. Growth factors are then protected and positioned in this scaffold. If RGTA is added before destruction by endocytosis, there is no room for it to bind because sites are saturated at the first application. Then growth factors positioned in the scaffold can be attracted by the RGTA "passing by" and carried away in the circulation. Particularly, the different ocular pain results may be attributed to two different designs of the studies.

Although we preferred performing CXL in one eye in all cases and selected another patient as the control, the authors performed CXL in both eyes and selected the fellow eye as the control. In bilateral CXL cases, it may have been difficult to discriminate levels of ocular pain between the eyes. Although the pain relief activity of RGTA has been documented in patients in both ophthalmology and skin healing,[11,12] the mechanism could not be understood at a molecular level. RGTA can indirectly help soothe the pain and discomfort because it promotes corneal healing.

There are also some limitations of the study, which mainly focused on the closure of corneal epithelial defects and did not provide a detailed analysis of other signs of corneal wound healing. For instance, whereas some patients revealed a complete closure of corneal epithelial defect with much smoother cornea without any punctual staining, others revealed complete closure with either linear healing line or punctual staining (**Figure 3**). In this study, the impact of topical RGTA eye drops on micro-architecture of corneal epithelialization was not investigated.

As supported by our findings after epi-off CXL, instillation of RGTA eye drops just before contact lens fitting appears to be a promising agent for promoting corneal epithelialization, controlling inflammation, and reducing ocular discomfort. The possible effects on corneal inflammation, haze formation, and other complications of CXL should be addressed in a larger population in a long-term prospective controlled trial.

AUTHOR CONTRIBUTIONS

Study concept and design (KG, SK); data collection (KG); analysis and interpretation of data (KG, MGG, SHMM, DB); writing the manuscript (KG, SK); critical revision of the manuscript (KG, MGG, SHMM, SK, DB); statistical expertise (KG); administrative, technical, or material support (KG); supervision (KG, SK, DB)

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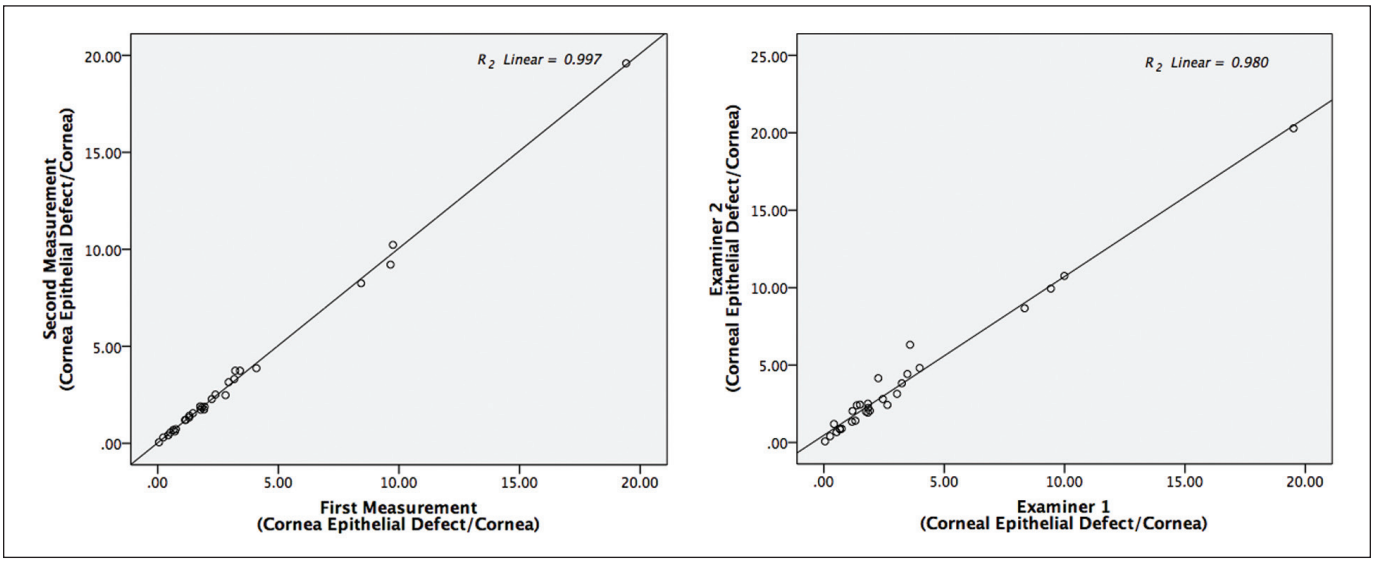

Figure A. These correlation plots represent intraobserver and interobserver repeatability of the measurement of the ratio (corneal epithelial defect/whole cornea) using the image analysis technique.

TABLE A

Corneal Topographic Characteristics of Study Groups

Parameter	RGTA (n = 30)	Control (n = 30)	*P*
Sim K1 (D)	46.3 ± 2.7	45.0 ± 2.9	.064
Sim K2 (D)	49.1 ± 3.3	48.5 ± 2.9	.443
K_{avg}	47.7 ± 2.9	46.7 ± 2.8	.182
K_{max}	54.5 ± 5.0	55.2 ± 4.8	.617
BCV	2.95 ± 1.6	2.87 ± 1.4	.847
Thinnest pachymetry (μm)	448.9 ± 33.6	452.7 ± 35.7	.676

RGTA = ReGeneraTing Agent; Sim K1 = simulated keratometry 1; D = diopters; Sim K2 = simulated keratometry 2; K_{avg} = average keratometry; K_{max} = maximum keratometry; BCV = Baiocchi Calossi Versaci index