Effect of the Regenerative Agent Poly (Carboxymethylglucose Sulfate) on Corneal Wound Healing After Corneal Cross-Linking for Keratoconus

George D. Kymionis, MD, PhD,† Dimitrios A. Liakopoulos, MD, MSc,* Michael A. Grentzelos, MD,* Konstantinos I. Tsoulnaras, MD,* Efstathios T. Detorakis, MD, PhD,* Béatrice Cochener, MD, PhD,‡ and Miltiadis K. Tsilimbaris, MD, PhD**

Purpose: To evaluate the effect of a regenerative agent (RGTA) [Cacicol20—poly(carboxymethyl glucose sulfate); OTR3, Paris, France] on corneal reepithelialization and pain after corneal cross-linking (CXL) for keratoconus.

Methods: In this prospective comparative (contralateral) clinical study, patients with bilateral progressive keratoconus underwent CXL treatment. The corneal epithelium during CXL was removed using transepithelial phototherapeutic keratectomy (Cretan protocol). One eye of each patient was randomly instilled with an RGTA (Cacicol20) once a day (study group), whereas the fellow eye was instilled with artificial tears (control group). Patients were examined daily until complete reepithelialization. Postoperative examinations included slit-lamp biomicroscopy to assess the epithelial defect size and subjective evaluation of pain.

Results: The study enrolled 18 patients (36 eyes). The mean epithelial defect size for study and control groups was 19.6 ± 4.2 mm^2 versus 21.5 ± 2.8 mm^2, respectively, at day 1 ($P = 0.019$) and 6.4 ± 3.4 mm^2 versus 7.9 ± 4.3 mm^2, respectively, at day 2 ($P = 0.014$). At day 3 postoperatively, 61.1% of study eyes were fully reepithelialized, compared with 11.1% of control eyes ($P = 0.002$).

Conclusions: RGTA (Cacicol20) instillation seems to result in faster corneal reepithelialization after CXL in this study. However, there was no significant effect in subjective pain/discomfort.

Key Words: regenerative agent, RGTA, Cacicol20, cornea, cross-linking, CXL

(*Cornea* 2015;34:928–931)

Received for publication February 15, 2015; revision received April 9, 2015; accepted April 10, 2015. Published online ahead of print June 9, 2015.

From the *Faculty of Medicine, Department of Ophthalmology, Vardinoyiannion Eye Institute of Crete (VEIC), University of Crete, Heraklion, Greece; †Department of Ophthalmology, Bascom Palmer Eye Institute, Miller School of Medicine, University of Miami, Miami, FL; and ‡Bâtiment 4 bis, Department of Ophthalmology, University Hospital, Brest, France.

B. Cochener and G. D. Kymionis are clinical investigators for Thea Laboratories, Inc, Clermont-Ferrand, France. The other authors have no funding or conflicts of interest to disclose.

Reprints: George D. Kymionis, MD, PhD, Vardinoyiannion Eye Institute of Crete (VEIC), University of Crete, Faculty of Medicine, Heraklion, Crete 71003, Greece (e-mail: kymionis@med.uoc.gr).

Corneal cross-linking (CXL) is a surgical technique used to strengthen ectatic cornea tissue, involving epithelial debridement and application of riboflavin and ultraviolet-A irradiation (UVA).[1–4] The standard CXL protocol requires corneal epithelial removal, which retains the drawback of postoperative pain and the risk of epithelium-related complications such as infection and recurrent erosions.[4]

Cacicol20 [poly(carboxymethyl glucose sulfate); OTR3, Paris, France] is a regenerative agent (RGTA) that has provided encouraging results in corneal lesions such as neurotrophic ulcers, chronic neurotrophic keratitis, and persistent epithelial defects.[5–8] The agent contains carboxymethyl dextran sulfate polymer, engineered to replace heparan sulfates, and seems to be effective in enhancing reepithelialization, restoring extracellular matrix architecture, and reducing pain.[5,6] These effects would be beneficial to eliminate the drawbacks of the epithelium-off CXL protocol. Rapid corneal reepithelialization after CXL has a key role in minimizing possible complications related to corneal wound healing.

The purpose of this study was to evaluate the efficacy of the RGTA (Cacicol20) in corneal reepithelialization and postoperative pain by comparing the epithelial defect size and the level of pain between the study eye (instillation of the RGTA) and control eye (instillation of artificial tears) in a series of patients with keratoconus who underwent bilateral CXL treatment.

PATIENTS AND METHODS

In this prospective comparative (contralateral) study, patients with progressive keratoconus were enrolled. The clinical diagnosis of keratoconus was based on corneal topography data (iTrace; Tracey Technologies, Houston, TX). Inclusion criteria were progressive keratoconus, patient age >18 years, corneal thickness >400 μm, no other ocular pathological signs, and no pregnancy or lactation. Keratoconus was graded with the Amsler–Krumeich classification; only patients with grade I and II were included in this study. Keratoconus was described as progressive when there had been an increase in the cone apex keratometry (K) of 0.75 diopter (D) or an alteration of 0.75 D in the spherical equivalent refraction in the previous 6 months.

All patients underwent bilateral CXL treatment according to the Cretan protocol.[9] One eye of each patient was

randomly instilled with an RGTA (Cacicol20) (study eye; 1 drop daily until complete reepithelialization), whereas the fellow eye was instilled with artificial tears (control eye; 1 drop daily until complete reepithelialization) in addition to the standard postoperative medication. The patient and the ophthalmologist who performed the postoperative examinations were masked to which eye the RGTA or artificial tears were instilled.

All patients were appropriately informed before their participation in the study and gave written informed consent. The research conformed to the tenets of the Declaration of Helsinki and the Institutional Review Board.

Surgical Technique

All procedures were performed in our institute by the same surgeon (G.D.K.) under sterile conditions. After administration of topical anesthesia comprising proxymetacaine hydrochloride 0.5% eyedrops (Alcaine; Alcon Laboratories, Inc, Fort Worth, TX), the corneal epithelium was removed by transepithelial phototherapeutic keratectomy (PTK) using the Allegretto WaveLight excimer laser (WaveLight Laser Technologie AG). Transepithelial PTK ablation was performed at a 7.0-mm zone at an intended depth of 45 μm. After epithelial removal, riboflavin (0.1% solution of 10 mg riboflavin-5-phosphate in 10 mL dextran-T-500 20% solution; Medicross, Medio-Haus, Behrensbrook, Neudorf, Germany) was instilled on the center of the cornea every 3 minutes for approximately 30 minutes. Ultraviolet A (UVA) irradiation was performed using a UVA optical system (CCL-365; Peschke Meditrade GmbH). Before treatment, an intended irradiance of 9.0 mW/cm^2 was calibrated using the UVA light meter YK-34UV (Lutron Electronic; Enterprise Co, Ltd, Taipei, Taiwan), which is supplied with the UV-X device. Irradiance was performed for 10 minutes, corresponding to a total surface dose of 5.4 J/cm^2. During UVA irradiation, riboflavin solution was applied every 3 minutes to maintain corneal saturation with riboflavin. At the end of the procedure, a silicone–hydrogel bandage contact lens (BCL) (Lotrafilcon B, Air Optix; Ciba Vision, Duluth; GA) was applied until full reepithelialization.

Postoperative Medication

Postoperative medication included ofloxacin (Exocin; Allergan Pharmaceuticals Ltd) 4 times daily and chloramphenicol/dexamethasone drops (Dispersadron; Thea Laboratories, Inc) 4 times daily in both eyes until the removal of the BCL. Patients were encouraged to use artificial tears at least 6 times per day. In addition to the standard early postoperative medication, the study eye was also instilled with Cacicol20 (one drop daily), whereas the fellow eye was instilled with artificial tears (one drop daily) until complete reepithelialization.

Postoperative Follow-up

All patients were examined daily until complete reepithelialization. Each postoperative daily examination included slit-lamp biomicroscopy to objectively assess epithelial healing (epithelial defect size). An ophthalmologist (unaware of which eye was instilled with RGTAs or artificial tears) performed the assessments. The epithelial defect size was calculated using the mean radius of the long and short axes of the remaining area. Other researchers have used this method to determine the remaining epithelial defect size.[10] After full reepithelialization, the BCL was removed and fluorescein was instilled to confirm the absence of an epithelial defect.

Subjective evaluation of pain (discomfort) was recorded on every postoperative day until complete reepithelialization. The pain score was evaluated on a scale of 0 to 4 as follows: 0 = no discomfort or pain; 1 = mild discomfort; 2 = moderate burning pain; 3 = burning pain requiring oral medication; 4 = severe constant or sharp pain not mitigated with oral medication.

Statistical Analysis

All data were collected in an Excel spreadsheet (Microsoft, Redmond, WA). SPSS software for Windows version 20 (SPSS, Inc, Chicago, IL) was used for statistical analysis of the results. Continuous variables are presented as mean ± SD. $P < 0.05$ is considered statistically significant. A sample size analysis (G*Power 3.1.3; Franz Paul, Universität Kiel, Germany) was performed for a 2-tailed t test with a large difference effect size, with a statistical power of 85% and a statistical significance level of 0.05, resulting in 18 patients participating in this prospective comparative (contralateral) study. Subjective and objective outcomes were compared using the paired Student t test and χ^2 test, where appropriate.

RESULTS

Thirty-six eyes of 18 patients (14 men and 4 women) were included in this prospective comparative (contralateral) study. Mean patient age was 24.1 ± 4.4 years (range, 18–34 years). No intraoperative or early postoperative complications were observed in any of the patients.

Eyes of the study group had smaller areas of epithelial defects than did the eyes of the control group at all postoperative follow-up visits. In particular, statistically significant differences were observed for epithelial defect size measurements between the study group (RGTA; Cacicol20) and control group (artificial tears) at day 1 (study group: 19.6 ± 4.2 mm^2 versus control group: 21.5 ± 2.8 mm^2; $P = 0.019$) and day 2 (study group: 6.4 ± 3.4 mm^2 vs. control group: 7.9 ± 4.3 mm^2; $P = 0.014$) postoperatively (Fig. 1).

At day 3 postoperatively, 61.1% (11/18) of eyes of the study group were fully reepithelialized, compared with 11.1% (2/18) of eyes of the control group ($\chi^2 = 9.753$, $P = 0.002$). At day 4 postoperatively, 100% (18/18) of eyes of the study group were fully reepithelialized, compared with 77.8% (14/18) of eyes of the control group. In 13 patients, the study eye healed 1 day earlier and in 1 patient 2 days earlier than the control contralateral eye. There was no statistically significant difference between the study and control groups in the pain index in any postoperative day ($P > 0.05$; Fig. 2).

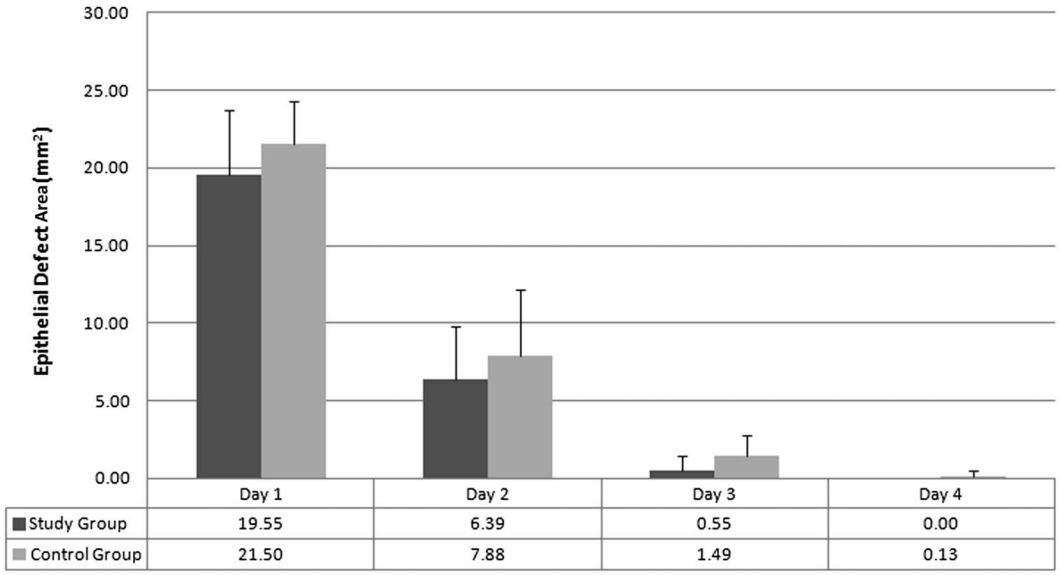

FIGURE 1. Plot of the mean epithelial defect size at all postoperative days until full reepithelialization. The study group reveals beneficial healing from the first postoperative day. The error bars represent ±1 SD.

DISCUSSION

The standard CXL protocol requires corneal epithelial removal.[2,4] Rapid corneal wound healing (reepithelialization process) is an important factor in minimizing the risk of epithelium-related complications such as infection and recurrent erosions and postoperative pain.[4]

The RGTA (Cacicol20) is a large biopolymer that binds matrix proteins that affect corneal healing.[11] The molecular activity of RGTAs has been described to involve the imitation of heparan sulfate molecules.[5,11] The RGTA retrieves the role of these molecules, restoring the extracellular microenvironment and improving the production of signals and essential growth factors for tissue healing.[11]

Previous studies have demonstrated the safety and efficacy of the RGTA (Cacicol20) in restoring several lesions of the corneal surface. The RGTA has already been used as a monotherapy for neurotrophic corneal ulcers and chronic neurotrophic keratitis providing encouraging results.[5–7] In our recent study, the combination of the RGTA (Cacicol20) with a BCL has been proved an efficacious therapeutic approach for persistent epithelial defects (unresponsive to conventional treatments for 4–8 weeks).[8] Contact lenses have shown beneficial effects on epithelial defects, and their combined application with the RGTA (Cacicol20) revealed effective results, because all patients had a short period of corneal epithelial healing (4–21 days).[8,10] Daily application had a favorable tolerance, and no side effects were noted.[8] Moreover, the integrity of the corneal epithelium remained, and no recurrence occurred during the follow-up period.[8]

In this study, the RGTA (Cacicol20) seemed to have a beneficial role in epithelial healing on most of the eyes of the study group compared with the eyes of the control group, during the reepithelialization process at the early postoperative period after CXL. Eyes instilled with the RGTA (Caciol20) (study group) had smaller areas of epithelial defects than did the eyes instilled with artificial tears (control

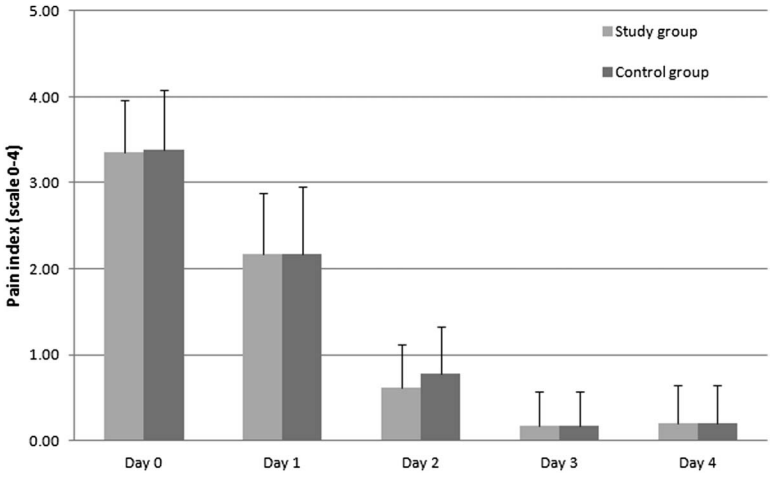

FIGURE 2. Plot of the mean subjective pain index at all postoperative days. Both groups show a similar evaluation of pain levels. The error bars represent ±1 SD.

group) on postoperative days 1 and 2. At postoperative day 3, 61.1% of eyes of the study group were fully reepithelialized compared with 11.1% of eyes of the control group (P = 0.002). At postoperative day 4, all eyes of the study group were fully reepithelialized compared with 77.8% of eyes of the control group. However, the statistically significant differences between the 2 groups regarding the epithelial defect size and time of complete reepithelialization do not necessarily imply strong clinical significance. Regarding the evaluation of pain, no significant difference was observed between the 2 groups at any postoperative day. This aspect is possibly biased, because of the use of contact lenses postoperatively, in both control and study groups.

A possible explanation of our findings could be a potential role of the RGTA (Cacicol20) in structural and cellular alterations occurring in the immediate period after the CXL procedure. In the irradiated area, stromal edema and a mild inflammatory reaction have been described as early responses to riboflavin and UVA irradiation.[12,13] Histological studies have shown several effects of the RGTA (Cacicol20) including amelioration of tissue edema, fibrosis, and inflammation, thereby improving wound repair.[14,15] Our study findings indicate that the RGTA (Cacicol20) could possibly promote–accelerate the reepithelialization process after CXL treatment. Moreover, this is the first study that compares the RGTA (Cacicol20) effect on nonpathological corneal epithelium, in addition to previously published articles that described the effect of the agent in pathological conditions such as neurotrophic ulcers and PEDs.[5,6,8]

In conclusion, the results of our study suggest that the addition of the RGTA (Cacicol20) to the standard postoperative medication after CXL could be an effective approach to enhance the epithelial recovery. To the best of our knowledge, this is the first clinical study evaluating the possible potential effect of the RGTA (Cacicol20) on corneal reepithelialization after CXL treatment. Larger patient series are necessary to evaluate the efficacy of this treatment protocol in clinical practice.

REFERENCES

1. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol.* 2003;135:620–627.
2. Raiskup-Wolf F, Hoyer A, Spoerl E, et al. Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: long-term results. *J Cataract Refract Surg.* 2008;34:796–801.
3. Kymionis GD, Grentzelos MA, Liakopoulos DA, et al. Long-term follow-up of corneal collagen cross-linking for keratoconus-the Cretan study. *Cornea*. 2014;33:1071–1079.
4. Filippello M, Stagni E, O'Brart D. Transepithelial corneal collagen crosslinking: bilateral study. *J Cataract Refract Surg.* 2012;38:283–291.
5. Chebbi CK, Kichenin K, Amar N, et al. Pilot study of a new matrix therapy agent (RGTA OTRA4120) in treatment-resistant corneal ulcers and corneal dystrophy [in French]. *J Fr Ophtalmol.* 2008;31:465–471.
6. Aifa A, Gueudry J, Portmann A, et al. Topical treatment with a new matrix therapy agent (RGTA) for the treatment of corneal neurotrophic ulcers. *Invest Ophthalmol Vis Sci.* 2012;53:8181–8185.
7. De Monchy I, Labbé A, Pogorzalek N, et al. Management of herpes zoster neurotrophic ulcer using a new matrix therapy agent (RGTA): a case report [in French]. *J Fr Ophtalmol.* 2012;35:187.e1–6.
8. Kymionis GD, Liakopoulos DA, Grentzelos MA, et al. Combined topical application of a regenerative agent with a bandage contact lens for the treatment of persistent epithelial defects. *Cornea*. 2014;33:868–872.
9. Kymionis GD, Grentzelos MA, Kankariya VP, et al. Long-term results of combined transepithelial phototherapeutic keratectomy and corneal collagen crosslinking for keratoconus: cretan protocol. *J Cataract Refract Surg.* 2014;40:1439–1445.
10. Grentzelos MA, Plainis S, Astyrakakis NI, et al. Efficacy of 2 types of silicone hydrogel bandage contact lenses after photorefractive keratectomy. *J Cataract Refract Surg.* 2009;35:2103–2108.
11. Barritault D, Caruelle JP. Regenerating agents (RGTAs): a new therapeutic approach [in French]. *Ann Pharm Fr.* 2006;64:135–144.
12. Wollensak G, Iomdina E, Dittert DD, et al. Wound healing in the rabbit cornea after corneal collagen cross-linking with riboflavin and UVA. *Cornea*. 2007;26:600–605.
13. Raiskup F, Spoerl E. Corneal crosslinking with riboflavin and ultraviolet A. I. Principles. *Ocul Surf.* 2013;11:65–74.
14. Brignole-Baudouin F, Warnet JM, Barritault D, et al. RGTA-based matrix therapy in severe experimental corneal lesions: safety and efficacy studies. *J Fr Ophtalmol.* 2013;36:740–747.
15. Cejkova J, Olmiere C, Cejka C, et al. The healing of alkali-injured cornea is stimulated by a novel matrix regenerating agent (RGTA, CACICOL20): a biopolymer mimicking heparan sulfates reducing proteolytic, oxidative and nitrosative damage. *Histol Histopathol.* 2014;29:457–478.