Parasurgical therapy for keratoconus by riboflavin–ultraviolet type A rays induced cross-linking of corneal collagen

Preliminary refractive results in an Italian study

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PURPOSE: To assess the effectiveness of riboflavin–ultraviolet type A rays induced cross-linking of corneal collagen in reducing progression of keratoconus and in improving visual acuity in patients with progressive keratoconus.

SETTING: Department of Ophthalmology, Siena University, Siena, Italy.

METHODS: This was a second-phase prospective nonrandomized open study. Starting in September 2004, 10 eyes of 10 patients (mean age 31.4 years) with bilateral keratoconus were treated by combined riboflavin–ultraviolet type A rays (UVA) collagen cross-linking. Radiant energy was 3 mW/cm^2 or 5.4 joule/cm^2 for a 30-minute exposure at 1 cm from the corneal apex. A complete ophthalmologic examination (uncorrected visual acuity [UCVA], sphere spectacles corrected visual acuity (SSCVA), best spectacle-corrected visual acuity [BSCVA]) was performed. Patients had corneal computerized topographic examination, linear scan optical tomography, endothelial cell count, ultrasound pachometry, intraocular pressure (IOP) evaluation, and HRT II system confocal microscopy at 1, 2, 3, and 6 months. After treatment, eyes were medicated and dressed with a soft contact lens.

RESULTS: Comparative preoperative and postoperative results showed increases of 3.6 lines for UCVA ($P = .0000112$), 1.85 lines for SSCVA ($P = .00065$), and 1.66 lines for BSCVA ($P = .00071$). Topographic analysis showed a mean K reduction of 2.1 $\pm$ 0.13 diopters (D) in the central 3.0 mm. Statistical analysis of IOP and endothelial cell count did not show significant differences. Topo-aberrometric analysis findings of corneal symmetry showed a trend toward increasing corneal symmetry with a major reduction in asymmetry between vertical hemimeridians.

CONCLUSIONS: Refractive results showed a reduction of about 2.5 D in the mean spherical equivalent, topographically confirmed by the reduction in mean K. Results of surface aberrometric analysis showed improvement in morphologic symmetry with a significant reduction in comatic aberrations.

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Keratoconus is a degenerative noninflammatory disease of the cornea with onset generally at puberty. It is progressive in 20% of cases and can be treated by lamellar or perforating keratoplasty. Its incidence in the general population is reported to be about 1 in 2000.[1] Incidences of 1 in 600 to 1 in 420 seem more in keeping with current diagnostic capacity. Changes in corneal collagen structure,[2,3] organization,[4] and intercellular matrix[5] as well as apoptosis[6] and necrosis of keratinocytes[7](Figure 1), prevalently or exclusively involving the central anterior stroma and the Bowman's lamina, are documented in the literature.[4–8] These findings are evidence of structurally weakened corneal tissue typical of keratoconus.

The technique of corneal collagen cross-linking consists of photopolymerization of stromal fibers by the combined action of a photosensitizing substance (riboflavin or vitamin B2) and ultraviolet type A rays (UVA) from a solid-state UVA source.[9] Photopolymerization increases the rigidity of corneal collagen and its resistance to keratectasia.[10]

The first studies in photobiology began in the early 1990s, with attempts to identify biological glues that could be activated by heat or light to increase the resistance of

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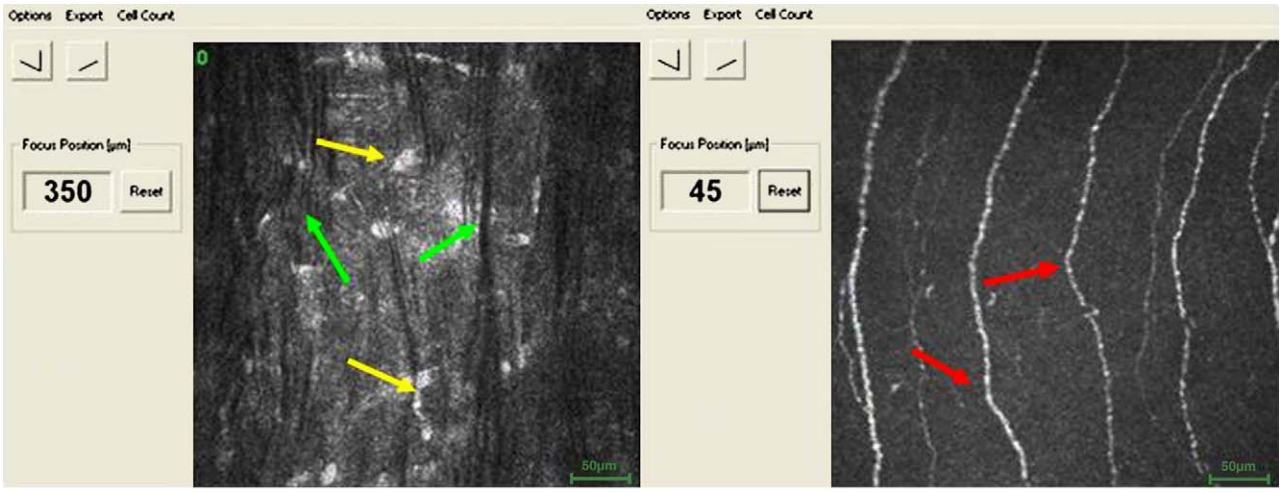

Figure 1. *A*: Dark microstriae (*green arrows*) and activated keratocytes (*yellow arrows*) in posterior stroma 350 µm in progressive keratoconus. *B*: Stretching and granular (necklace pearls) aspect of nervous fibers (*red arrows*) in progressive keratoconus, anterior subepithelial stroma (45 µm), in vivo HRT II confocal microscopy.

stromal collagen.[11] It was discovered that the gluing effect was mediated by an oxidative mechanism associated with hydroxyl radical release. A similar mechanism of hardening and thickening of collagen fibers has been shown in corneal aging[12] and is related to active glycosylation of age-dependent tropocollagen molecules.

The idea to use this conservative approach to treat keratoconus was conceived in Germany in the 1990s by a research group at Dresden Technical University.[9] The aim was to slow or arrest progression to delay or avoid recourse to perforating keratoplasty. The basis for its use finds clinical and scientific support in the fact that young diabetic patients never have keratoconus and in the few exceptions, it predated the onset of diabetes and did not progress due to the natural cross-linking effect of glucose, which increases corneal resistance in these patients.

The biomechanical properties of the cornea depend on the characteristics of collagen fibers,[2,3] interfibril bonds,[5] and their spatial-structural disposition.[4] The biomechanical resistance of the cornea of keratoconus patients is half the normal value. The technique of corneal collagen cross-linking has been used experimentally (Dresden, Zurich, Siena) to at least temporarily block progression of keratoconus in the refractive phase. Collagen turnover is about 2 to 3 years. Cross-linking "freezes" stromal collagen, increasing the biomechanical stability of the cornea.[13]

The method of corneal cross-linking using riboflavin and UV light is technically simple and less invasive than all other therapies proposed for keratoconus, and unlike other mini-invasive methods, such as intrastromal rings (INTACS) and excimer laser surgery, that do not block keratectasia but merely treat the refractive effects of the disease, it treats and prevents the underlying pathophysiological mechanism.[9]

The main aim of this study was to assess the effectiveness and safety of riboflavin-UV induced cross-linking of corneal collagen in reducing the progression of keratoconus and in improving visual acuity. This is the first Italian second-phase prospective nonrandomized open study. It comprised 10 patients with progressive keratoconus.

PATIENTS AND METHODS

The study was approved by the ethics committee of Siena University under the principles of the Helsinki declaration; informed specific consent was previously (minimum 10 days) obtained from all participants. Beginning in September 2004, 10 eyes (6 right, 4 left) of 10 patients (2 women, 8 men) with a mean age of 31.4 years (range 21 to 39 years) were selected and treated. In 2 patients, the fellow eye (left) had had perforating keratoplasty (2 and 3 years previously) and was not used as control. In the other 8 cases, the fellow eye was used as a control. All patients had bilateral keratoconus without subepithelial scarring and could not tolerate contact lenses. Vogt striae were detected by biomicroscopic examination in 5 eyes and were absent in the other 5. Five eyes were in Krumeich stage

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2 and 5 in stage 3. In the 8 fellow control eyes, no striae or scars were observed; 4 were in stage 2 and 4 in stage 1.

Before treatment, all patients had biomicroscope examination and assessment of uncorrected visual acuity (UCVA), best spectacle-corrected visual acuity (BSCVA), and intraocular pressure (IOP) (Tono-Pen II XL, Mentor); those with myopic ametropia also were assessed for sphere-corrected visual acuity (SSCVA). They also had corneal computerized topographic examination (Eye Top CSO V 7.1.1), linear scan optical tomography (Orbscan II B & L), endothelial cell count by noncontact endothelial microscope (Konan Keeler Non Con Robo), and ultrasound pachymetry (DGH Pachette 2). Patients also had laser confocal microscope examination (Heidelberg HRT II). All examinations were repeated 1, 2, 3, and 6 months after cross-linking treatment.

The surgical procedure (Figure 2) consisted of topical anesthesia (instillation of lidocaine 4% 3 times in 15 minutes), placing the patient under the operating microscope, and inserting a lid speculum with closed valves and screw regulation (S.I.R. Ophthalmic 812T). A 7.0 mm circle was traced on the epithelium with a Thornton corneal marker (Asico AE2710) in which the epithelium was removed with a blunt spatula (Asico AE2766). This was followed by instillation of 2 to 4 drops of a solution containing riboflavin 0.1% and 20% dextran (500,000 Da) prepared immediately before the operation and placed in a 1 mL syringe that was left in place for 5 minutes before beginning UV exposure. After instilling another 2 to 4 drops, the UV lamp was turned on. The UV source was a solid-state experimental device (Exerion-Sas) consisting of 2 UV LEDs (370-10 TO 46-ball lens, 750 mW, Roithner Lasertechnic) with a potentiometric voltage regulator (Figure 3). Irradiated energy was controlled by a UV power meter (Lasermate Q-Coherent). The source was focused on the apex of the cornea, distance 10 to 12 mm, to obtain a radiant energy of 3 mW/cm^2 or 5.4 J/cm^2 for 5 minutes. The lamp was then turned off, the riboflavin–dextran solution was again instilled, and the 5-minute exposure repeated 5 times (total exposure 25 minutes, total treatment 30 minutes). After treatment, the eye surface was washed with 20 mL balanced salt solution, medicated with 2 to 4 drops of ofloxacin (Exocin) and 2 to 4 drops of cyclopentolate (Ciclolux) and dressed with a soft contact lens (Schalkon SofClear +0.5 diopters [D]).

Patients received paracetamol–codeine (500 mg to 30 mg) every 8 hours and the postoperative medication was repeated 4 times per day until removal of the contact lens on day 5. Biomicroscopic examination was then performed to assess epithelial repair. Topical therapy with flurbiprofen eyedrops (Ocufen, 4 times per day) and ofloxacin eyedrops (Exocin, 4 times per day) was continued for a further 20 days.

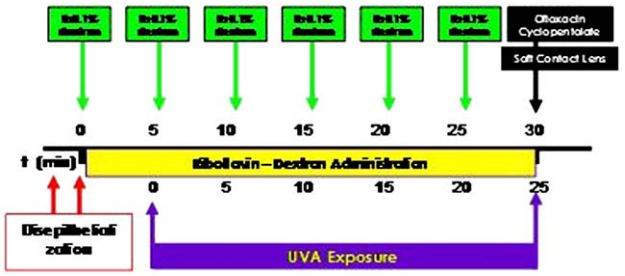


Figure 2. Summary of surgical procedure and timing of UVA (violet) and riboflavin-Dextran administration.

Topographic assessment was performed with axial (step 0.75/1 diopter (D)) and tangential (step 1.5 D) algorithms. Altimetric evaluation was also performed using an aspherotoric reference surface located tangentially to the geometric center of the cornea, scale 5 μm, reducing dioptric power of the reference sphere by 10 D so as to highlight the localization and elevation of the apical region. After removal of the topographic-revealed pupil, aberrometric analysis was performed of the corneal wavefront (Seidel) with a 5.0 mm simulated entrance pupil (Figure 4). Orbscan examination was only used for anterior elevation parameters because posterior elevations and pachymetry were impeded by postoperative subedema. The numerical data extrapolated from all procedures were entered in a data sheet (MS Excel XP) and analyzed with GraphPad Prism 3.0.

RESULTS

Mean refraction for the best visual acuity before treatment was −2.85 D spherical (+1.00/−8.00) and −4.7 D cylindrical (−2.50/−6.50) with a mean spherical equivalent of −4.7 D (−0.75/−10.75) (Figure 5).

Mean UCVA was 1.3/10 (20/154 snellen lines), range 1/30 to 3/10 (20/600 to 20/66) and sphere corrected visual acuity (SSCVA) was 2.87/10 (20/70), range 1/10 to 5/10 (20/200 to 20/40). Mean BSCVA was 4.1/10 (20/48), range 2/10 to 6/10 (20/100 to 20/33) (Figure 6).

Mean central corneal thickness (CCT) before treatment measured by ultrasound pachymetry was 431.5 μm (range 406 to 468 μm), showing a small increment after treatment, presumably resulting from subedema (mean 463.3 μm; range 418 to 487 μm) that dropped to 450.6 μm (range 416 to 480 μm) at 3-month follow-up as edema cleared. Student *t* test for paired data did not find any significant difference between these values. Statistical analysis of IOP and endothelial cell count did not show significant differences between preoperative and postoperative data at all follow-up times. Subjective refraction showed a slow and continuous reduction in the follow-up: sphere value starting from a mean of −2.85 D ± 3.68 (SD) preoperatively, −1.95 ± 2.83 D at 1 month; −1.875 ± 2.56 D at 2 months, and −1.425 ± 2.54 at 3 months. Cylindric errors had a similar course: starting from a preoperative mean of −4.7 ± 1.27 D, decrease at the first month of −4.175 ± 1.29 D, −3.65 ± 1.35 D at the second month, and −3.5 ± 1.25 D at the end of the follow-up. Spherical equivalent value showed a decrease starting from −4.7 ± 3.35 D preoperatively, to −4.0375 ± 3.24 D at the first month, to −3.7 ± 3.18 D, and ending at −2.495 ± 3.06 D at third month of follow-up. Functional results included increases in UCVA and BCVA, preoperative BCVA identical to UCVA at 3 months, and increases of 3 Snellen lines in UCVA and 1.2 lines in BSCVA between preoperative and 1-month postoperative examinations, with statistical significance of $P = .00002$ for UCVA and $P = .00093$ for BSCVA (Mann-Whitney *U* test). Comparison of

Figure 3. *A*: Dresden modified Siena Tip with double 370/10 UV LEDs (Exerion Sas). *B*: Experimental electronic device for UV LEDs alimentation (Exerion Sas).

preoperative and 3-month follow-up data showed increases of 3.6 lines for UCVA ($P = .0000112$), 1.85 lines for SSCVA ($P = .00065$), and 1.66 lines for BSCVA ($P = .00071$). Topographic analysis with the axial refractive algorithm showed a mean reduction in dioptric power of 2.1 $\pm$ 0.13 D in the central 3.0 mm, with a reduction of 2.4 $\pm$ 0.16 D in the minimum value and 1.9 $\pm$ 0.08 D in the maximum value at 3 months, which is in line with the pilot study (Figure 7). Analysis of corneal symmetry based on automatic calculation of symmetry index by the topographer showed a trend toward increasing corneal symmetry (Figure 8) with a reduction in mean value from 6.263 before treatment to 4.258 at 3 months and a similar trend in all eyes analyzed from the second month.

A similar result emerged from analysis of hemimeridians, with a major reduction in asymmetry between

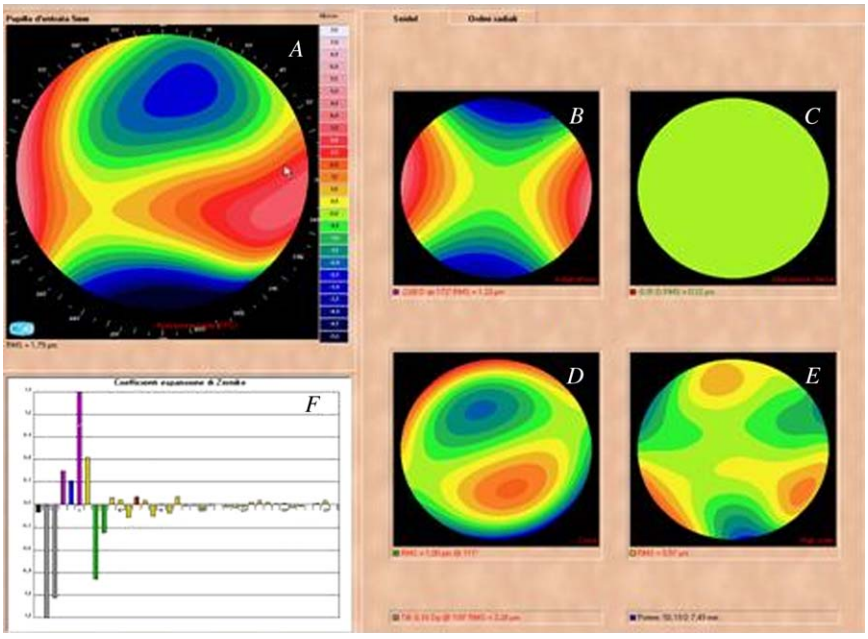


Figure 4. The CSO EyeTop Corneal Wavefront Seidel analysis of a 5.0 mm pupil in a left keratoconic eye. *A*: Total anterior optical path difference. *B*: Astigmatism deviation. *C*: Spherical aberration. *D*: Coma deviation. *E*: High-order (over 4th) aberration. The F barplotting of the single Zernike coefficients.

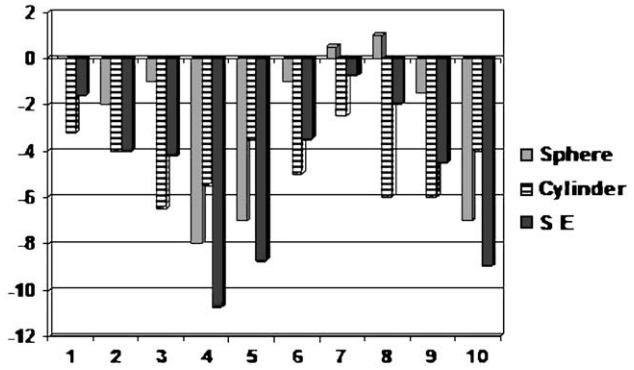


Figure 5. Preoperative refraction in the 10 treated eyes. Cylindric component was expressed in negative value.

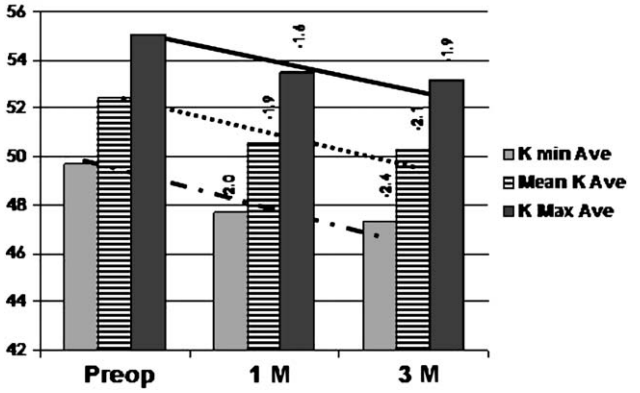


Figure 7. Topographic measured minimum, maximum, and mean K-readings at 3.0 mm. The lines over the bars indicate calculated linear regression.

vertical hemimeridians, leading to a significant reduction between preoperative and second month values (paired *t* test) and increasing significance from $P = .029$ at 2 months (significant with 95% confidence interval [CI]) to $P = .001$ at 3 months (99% CI). The reduction in difference between horizontal hemimeridians was also significant ($P = .033$) at 2 months, becoming highly significant at 3 months ($P = .0001$) (Figure 9). The reduction in asymmetry of horizontal and vertical hemimeridians was not significant at 1 month ($P = .11$ and $P = .39$, respectively). Anterior apical elevation, evaluated by corneal topography and Orbscan II, using as reference both the tangent at the corneal geometric center and best-fit sphere floating, showed a significant reduction that reproduced a similar proportional reduction in anterior elevation, although with different numbers (Figure 10). Statistical analysis of elevation parameters showed significant differences at 2 months with respect to before treatment ($P < .001$); this was maintained at 3 months with both analyses.

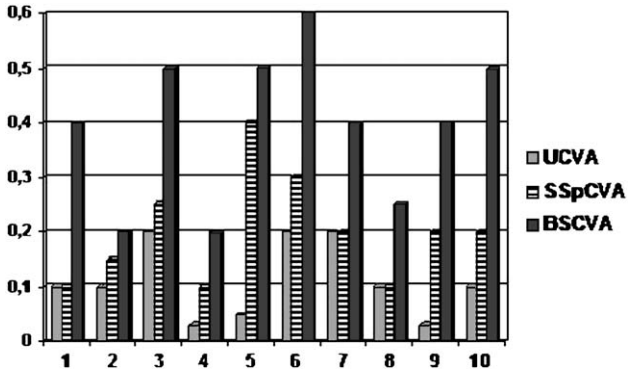


Figure 6. Preoperative visual acuity of the 10 treated eyes (decimal fraction).

Corneal wavefront surface aberrometry according to Seidel (Figure 11) showed a major reduction in root mean square, which was significant at 2 and 3 months and borderline significant at 1 month with respect to before the operation.

Spherical and higher-order aberrations did not show significant variations in the follow-up period, whereas the coma component showed a very significant reduction at 1 month with respect to before the operation and persisted throughout the follow-up period (Figure 12). In the fellow eye, a progression of keratoconus in 37.5% of the cases (3 of 8 eyes) was observed with increased corneal curvature, elevation and comatic aberrations, and decreased UCVA, SpCSVA, BSCVA, and pachymetric values. In the other 5 eyes (62.5%) topographic, aberrometric, and functional impairment was not observed.

DISCUSSION

Although the current study was limited in terms of follow-up and number of patients, it confirms that UV emitted by the light emitting diodes (LEDs) is perfectly calibrated in energy density to produce apoptosis, hence, necrosis of "unhealthy" activated keratocytes, in addition to being completely absorbed by riboflavin beyond the programmed dose and necessary thicknesses. The results of the pilot study of riboflavin–UV induced corneal collagen cross-linking were encouraging as far as safety and effectiveness are concerned. The procedure has few side effects for the cornea (no numerical or morphologic modifications of the endothelium) or for the posterior segment, where optical coherence tomography examination conducted preoperatively and 7 days and 3 months after treatment showed identical retinal macular and perimacular thicknesses. These 2 results, together with confocal microscope evidence of almost complete disappearance of keratinocytes

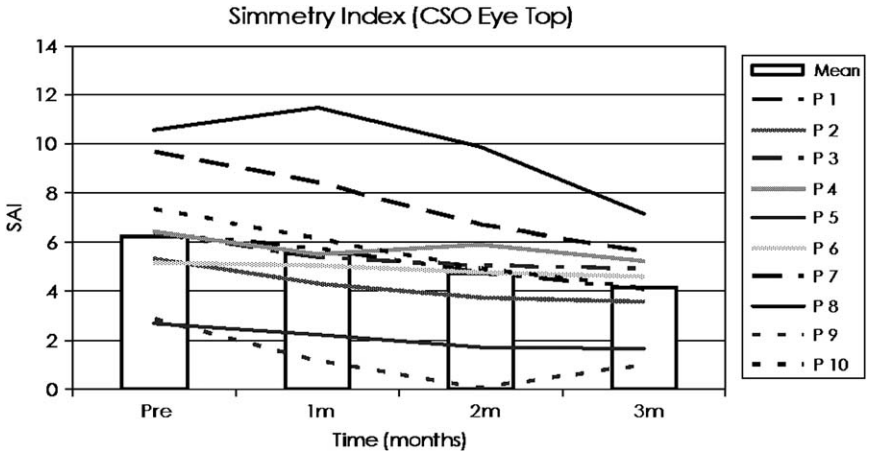
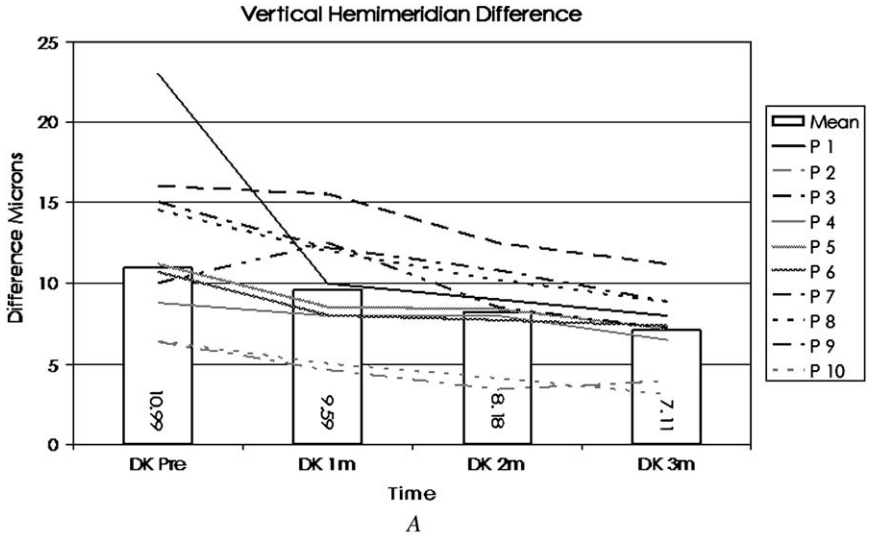
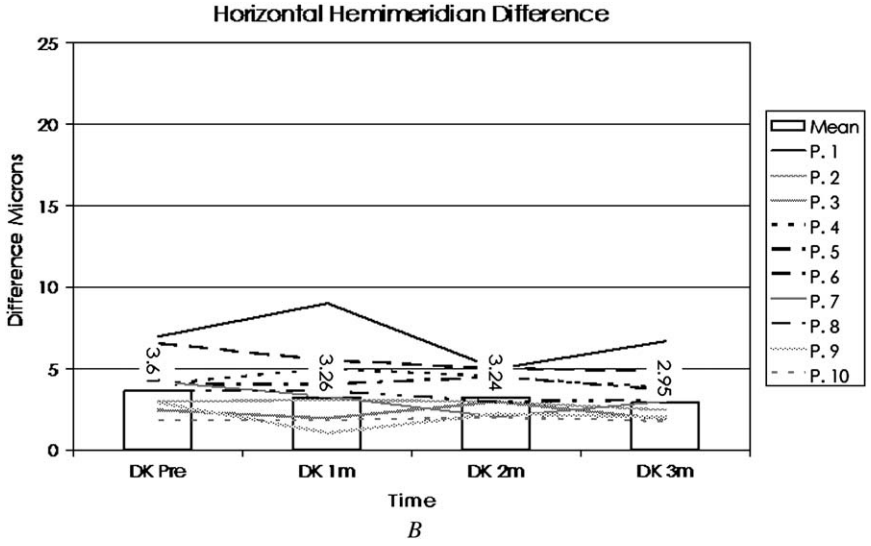


Figure 8. Topographic calculated symmetry index in the follow-up. The bars are the mean value, and the lines indicate the single cases.



A



B

Figure 9. Vertical (*A*) and horizontal (*B*) hemimeridian difference. Lines are the single cases and bars are the mean. A larger difference and a higher reduction in difference in vertical hemimeridian are evident; this significant reduction explains a significant reduction in the symmetry index. The horizontal hemimeridians do not have the same evolution.

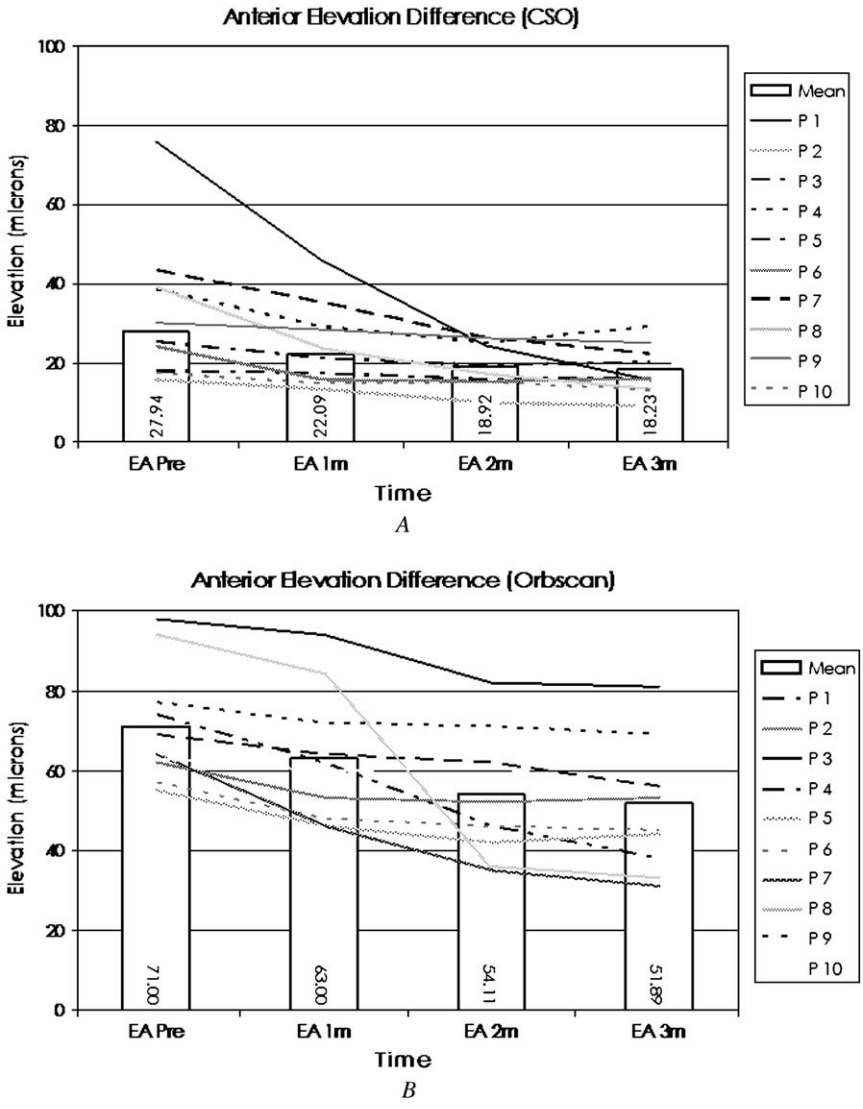


Figure 10. Anterior surface elevation with tangent aspherotoric surface reference 10 D reduced measured by CSO EyeTop corneal topography (*A*) and anterior surface elevation with floating best-fit sphere measured by Orbscan linear scansion tomography (*B*). The absolute value was different, but the same percentage of reduction was measured.

in the anterior 350 µm of the stroma and their presence at preoperative levels in areas immediately adjacent, confirm that UV emitted by the LEDs is perfectly calibrated in energy density to produce apoptosis and hence necrosis of "unhealthy" activated keratinocytes, besides being completely absorbed by riboflavin beyond the programmed dose and necessary thicknesses. Confocal microscope evidence of subclinical corneal edema only in areas devoid of keratinocytes indicates the significant degenerative effect of radiation and the lack of inflammatory phenomena, confirmed by the absence of inflammatory cells in treated and adjacent areas. Refractive results were similar to those obtained in Dresden pilot study concerning the reduction of about 2.5 D in the mean spherical equivalent, as confirmed by the reduction in mean curvature (K) detected topographically. This finding is significant and not worse than the results of much more invasive procedures[15,16] with complication rates of 20% to 30%, but this only in part explains the improved functional performance obtained in these 10 patients.[14–17] It seems logical that reduction in refractive defect is associated with a significant increase in UCVA (+3.6 Snellen lines), but the increase in SpCSVA and especially BSCVA (2 lines) is not easy to explain. Topographic and surface aberrometric analysis together with Orbscan, however, provide an insight: The improvement in morphologic symmetry reflected a significant reduction in coma aberrations at 1 month and a decrease in anisorefractance of the corneal hemimeridians, which means greater, necessarily symmetrical coherence between defect and correcting lens. Although our medium period refractive and functional results were in line with those in the pilot study, in our series improvement was

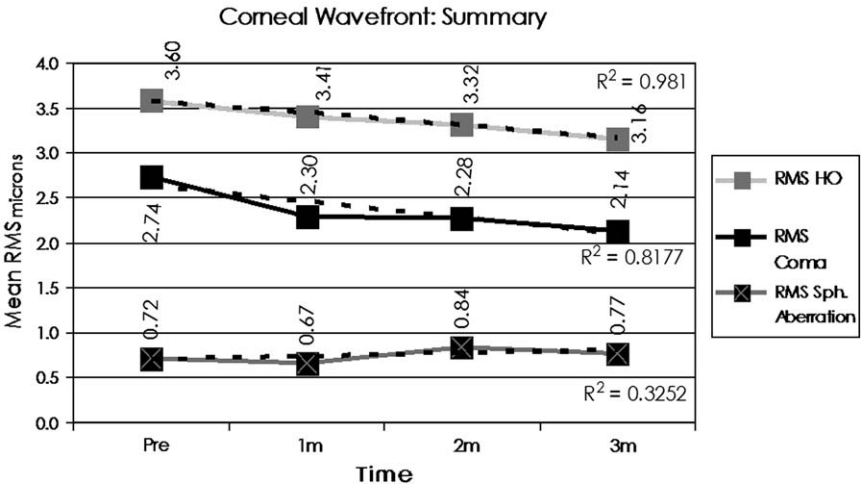


Figure 11. CSO EyeTop topographer Seidel corneal wavefront analysis with 5 mm simulated pupil, centration on geometric corneal center. Linear regression shows a significant, good correlation in HO RMS and coma RMS reduction between preoperative and postoperative data; the linear regression for Spherical aberration data show a not significant reduction in the follow-up.

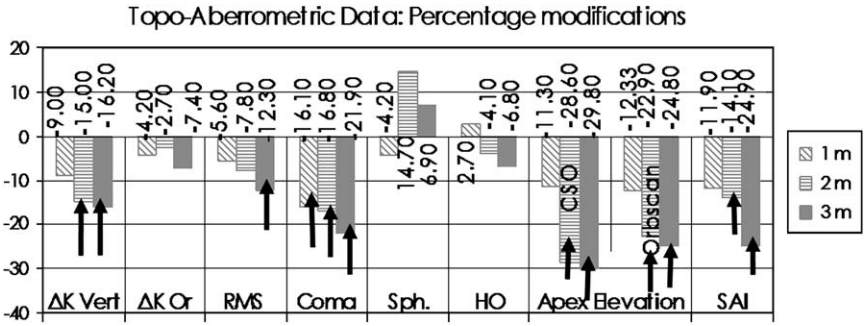


Figure 12. Percentage variations in analyzed keratotopographic parameters; red arrows indicate (paired *t* test) significant difference with preoperative data ($P < .01$).

already evident at the first postoperative examination, with significant differences in evolution of corneal K-readings, and particularly corneal form descriptors, against a latency of about 3 months in the pilot study. This difference could be caused by instrumentation and therapy. Indeed, the instrument used by us (Exerion S.A.S.) is powered by an electronic controller that stabilized the energy during UV treatment, eliminating the initial emission peak and the tail at the end of treatment that are typical of battery-powered devices. The constant voltage and energy of the supply system ensure constant intensity of emission by the LED over the entire exposure time. The other source of difference is the practice of dressing the cornea with a soft contact lens. This has positive effects on epithelial regrowth and on the initial stage of stromal rearrangement that cannot be obtained by simply bandaging the eye. Our preliminary data show that riboflavin–UV cross-linking is safe and effective to reduce the progression of keratoconus overall in early stages of the disease (first and second Krumeich stage), especially in young patients without Vogt striae. The optical and visual performance improvement seems to be particularly related to increasing corneal symmetry induced by restored corneal rigidity.

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