Penetration of riboflavin and postoperative pain in corneal collagen crosslinking

Excimer laser superficial versus mechanical full-thickness epithelial removal

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PURPOSE: To compare the severity of postoperative pain and the rate of penetration of riboflavin between eyes treated by corneal crosslinking (CXL) using excimer laser superficial epithelial removal and mechanical full-thickness epithelial removal.

SETTING: Departments of Ophthalmology, Ullevål University Hospital, Oslo, and University Hospital of Northern Norway, Tromsø, Norway.

METHODS: Patients with corneal ectasia were treated with superficial corneal epithelial removal using the excimer laser programmed to 35 μm of phototherapeutic keratectomy (Group 1) or mechanical full-thickness epithelial removal with a brush (Group 2). Pain was evaluated postoperatively by the patients' subjective evaluation and need for analgesia. The duration of topical application of riboflavin to achieve stromal saturation was measured.

RESULTS: Thirty eyes of 30 patients, 15 in each group, were treated. Postoperative pain was severe in 40.0% of patients in Group 1 and in no patient in Group 2 ($P = .009$) and moderate in 53.3% and 33.3%, respectively (no significant difference). The mean time to riboflavin saturation was 43.7 minutes $\pm$ 10.8 (SD) in Group 1 and 31.3 $\pm$ 3.0 minutes in Group 2 ($P = .001$).

CONCLUSION: Superficial epithelial removal using the excimer laser resulted in more postoperative pain and the need for prolonged application of riboflavin to achieve corneal saturation.

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Corneal collagen crosslinking (CXL) is a promising treatment for progressive corneal ectasia.[1–3] During treatment, riboflavin (vitamin B_2) and ultraviolet-A light (UVA) cause covalent bond formations between corneal collagen fibers. Adequate stromal saturation of riboflavin is essential to the efficacy of the CXL process and to the safety of more posterior parts of the eye during radiation.[2,4]

The tight junctions in the corneal epithelium act as a natural barrier to the penetration of large molecules. Thus, in the CXL method described by Wollensak et al.,[2] the epithelium is removed to allow sufficient penetration of riboflavin into the corneal stroma. The tight junctional complexes are found mostly between the superficial cells of the epithelium.[5] Our hypothesis was, therefore, that satisfactory riboflavin saturation might be achieved after removal of only the superficial 35 μm of the corneal epithelium with the excimer laser. We also wanted to determine whether preserving the basal epithelium would lead to less postoperative pain.

PATIENTS AND METHODS

Consecutive eyes of patients with diagnosed corneal ectasia were included in the study. The research followed the tenets of the Declaration of Helsinki. All patients provided written informed consent, and a local ethics committee (Tromsø, Norway) approved the study.

Standard examinations, including corneal topography and manifest refraction, were performed preoperatively and postoperatively. Except for epithelial removal, all patients were treated with CXL according to standard protocol.[2] The first patients (Group 1) were treated with superficial epithelial removal by excimer laser (iRES, Ligi), programmed to remove the anterior 35 μm of the corneal epithelium in a diameter of 8.0 mm using the phototherapeutic keratectomy mode. The next patients (Group 2) had full-thickness epithelial removal using the Amoils brush (Innovative Excimer Solutions, Inc.) in a diameter of 8.0 mm. After the superficial or full-thickness epithelial removal and after

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topical anesthesia of proparacaine hydrochloride was administered, riboflavin 0.1% solution (10 mg riboflavin-5-phosphate in 10 mL dextran-T-500 20.0% solution) was administered for a minimum of 30 minutes (1 drop per 5 minutes) until stromal saturation was confirmed by slitlamp examination (determined by the presence of riboflavin flare in the anterior chamber). The time for topical application of riboflavin to achieve stromal saturation was measured. The corneas were exposed to UVA radiation for 30 minutes using an UV-X lamp (Peschke, Meditrade GmbH). All treated eyes were dressed with a bandage contact lens (Night & Day, Ciba Vision).

Standard postoperative medications were paracetamol (acetaminophen) 500 mg every 8 hours for 5 days and topical dexamethasone and chloramphenicol 4 times per day for 3 weeks. All patients were also given 1 minim (0.5 mL) topical tetracaine after surgery. The patients were instructed to administer the tetracaine only in case of severe pain.

At the 1-week follow-up, postoperative pain was evaluated by the patients' subjective evaluation and their need for tetracaine. The patients' need for tetracaine was used as an indication of severe postoperative pain. Subjective severe pain without the need for tetracaine was classified as moderate pain.

The 2-sample *t* test was used to test for significant differences between the 2 groups. Backward stepwise logistic regression analysis was used to study correlations between data. Differences were considered significant if the *P* value was less than 0.05. Statistical analyses were performed using SPSS software (version 16.0, SPSS Inc).

RESULTS

Thirty consecutive eyes of 30 patients were evaluated. Group 1 and Group 2 each comprised 15 patients. Table 1 shows the patients' characteristics. The 2 groups were comparable in age, sex, and corneal thickness. There was, however, a statistically significant difference between groups in the maximum keratometry (K) value ($P = .01$). Logistic regression analysis did not show a correlation between the maximum K value and postoperative pain or the application time of riboflavin.

Table 1. Patient characteristics.

Group	Age (Y)	Sex (M/F)	CCT (μm)	Kmax (D)
Group 1				
Patient 1	33	M	435	53.6
Patient 2	49	M	429	48.7
Patient 3	43	M	417	51.6
Patient 4	28	M	438	50.1
Patient 5	25	M	436	51.4
Patient 6	23	M	494	55.9
Patient 7	18	M	447	53.4
Patient 8	18	M	484	49.0
Patient 9	33	M	435	50.3
Patient 10	33	M	548	40.3
Patient 11	41	M	430	50.4
Patient 12	37	M	500	47.7
Patient 13	25	F	460	43.6
Patient 14	22	M	450	53.3
Patient 15	40	M	443	52.6
Mean±SD	31.2 ± 9.5	14/1*	456 ± 35	50.1 ± 4.0
Group 2				
Patient 1	28	F	430	49.7
Patient 2	18	M	391	48.0
Patient 3	21	M	403	55.6
Patient 4	28	M	470	46.7
Patient 5	53	F	416	51.0
Patient 6	33	M	426	53.0
Patient 7	24	M	460	45.9
Patient 8	50	M	436	47.4
Patient 9	40	M	528	46.6
Patient 10	28	M	474	45.5
Patient 11	18	M	580	42.7
Patient 12	28	M	444	41.7
Patient 13	33	M	533	42.0
Patient 14	18	M	522	47.5
Patient 15	26	M	445	47.9
Mean±SD	29.7 ± 10.8	13/2*	464 ± 54	47.4 ± 3.9

CCT = central corneal thickness; Kmax = maximum keratometry value
*Numbers, not means

There was a statistically significant difference between groups in severe postoperative pain ($P = .009$). The postoperative pain was severe in 6 patients (40.0%) in Group 1 and no patient in Group 2 ($P = .009$). The pain was moderate in 9 patients (53.3%) and 5 patients (33.3%), respectively; the difference between groups was not statistically significant. The difference between groups in overall pain experience was highly statistically significant ($P = .0001$).

The mean time needed to achieve stromal saturation when applying riboflavin was 43.7 minutes ± 10.8 (SD) in Group 1 and 31.3 ± 3.0 minutes in Group 2; the difference between groups was statistically significant ($P = .001$). Multivariate analysis showed no correlation between the application time of riboflavin and postoperative pain.

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DISCUSSION

Epithelial cells adhere to each other through intercellular junctions consisting of gap junctions, desmosomes, adherens junctions, and tight junctions. The latter 3 junctions are often referred to as the epithelial junctional complex.[6] Tight junctions generally encircle cells at the apical end of the lateral membrane, and they form a paracellular diffusion barrier that regulates epithelial permeability to large molecules. The tight junctional complexes are found only between the superficial cells of the corneal epithelium.[7] It is therefore believed that to saturate the corneal stroma with riboflavin during CXL treatment, this barrier function of the epithelium must be broken. In the procedure described by Wollensak et al.,[2] epithelial removal by scraping is recommended to bypass the epithelial barrier. There has, however, been discussion about whether removal of the epithelium is necessary during CXL. Chan et al.[8] performed CXL with an intact epithelium. They report that this procedure was as effective and as safe as removing the epithelium. The CXL treatment, however, was performed immediately after insertion of an intrastromal ring segment, which caused an opening in the epithelial barrier. Pinelli and El Beltagi found that am intact epithelium did not significantly limit the penetration of riboflavin and that CXL treatment was equally efficient whether the epithelium was intact or removed (R. Pinelli, MD, T. El Beltagi, MD, "C3-R: the Present and the Future. You Can Leave the Epithelium Intact," Ophthalmology Times Europe, October 1, 2008, pages 13–15. Available at: http://www.iogen.fi/files/Ophthalmology_Times_Europe_-_C3-R__the_present_and_the_future.pdf. Accessed April 23, 2009).

In a study using porcine eyes,[9] the effects of CXL treatment on light transmission was evaluated after different protocols involving complete epithelial removal, scraping of the superficial epithelium with a scalpel (intact basal epithelium), or administration of topical tetracaine. The authors report that superficial epithelial trauma or tetracaine administrations were not sufficient to permit penetration of riboflavin into the corneal stroma and concluded that complete epithelial removal was necessary. However, concerns about whether these findings in porcine eyes are relevant to humans have been raised.[10]

In our study, the corneal stroma was saturated with riboflavin after removal of only the superficial layers of the epithelium; however, longer application of riboflavin was needed in these eyes than in the eyes that had full-thickness epithelial removal. We therefore believe that remaining basal epithelium is another relative barrier to the penetration of riboflavin.

We also hypothesized that not removing the basal epithelium would lead to less patient discomfort after the procedure. However, patients treated with partial epithelial removal had more severe pain than patients treated with full-thickness epithelial removal. Most sensory nerve receptors in the cornea are nociceptors, which result in the perception of pain when stimulated. These receptors usually have the lowest threshold for mechanical stimulation. The nerve fibers enter the cornea in the middle third of the stroma and run anteriorly and toward the central area in a radial fashion, giving rise to branches that innervate the anterior stroma. In the interface between Bowman layer and the anterior stroma, the stromal nerves form the subepithelial plexus. They then perforate Bowman layer and form the sub-basal epithelial nerve plexus. Nerve fibers from this plexus are known to be responsible for the innervation of the epithelium. Homogenous distribution of nerve endings across the cornea guarantees an efficient detection of external noxious stimuli, and injuries to individual epithelial cells may be sufficient to trigger pain perception.[11,12] Our finding of increased postoperative pain in the group treated with partial epithelial removal may be related to the preservation and exposure of these nerve endings.

Another possible explanation of the difference in postoperative pain could be related to the difference in treatment time and amount of riboflavin applied. However, we found no statistical evidence to support this theory.

The 2 groups in our study were well matched overall; however, there was a significant difference in maximum K values. This could indicate that Group 1 had more advanced keratoconus than Group 2, which again could have had an impact on our results. However, the corneal thickness was well matched between groups and multivariate analysis did not show a correlation between the maximum K value and postoperative pain or treatment time. We therefore believe our data are valid.

In our study, postoperative pain was assessed by the patients' subjective evaluations and their need for tetracaine. Pain is a subjective state that is difficult to evaluate objectively. The methodology most commonly used for evaluation of pain severity is the visual analogue scale (VAS). The VAS is, however, considered to be of most value when evaluating changes within individuals and of less value when comparing across a group at a single time point.[13]

In conclusion, in CXL treatment, superficial epithelial removal by excimer laser resulted in more postoperative pain than full-thickness removal. In addition, superficial laser ablation led to prolonged application of riboflavin to achieve corneal saturation.

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