CASE REPORTS

USING COLLAGEN CROSS-LINKING FOR THE TREATMENT OF CORNEAL DISEASES

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INTRODUCTION

Corneal collagen cross-linking with riboflavin and UVA - light is a new therapeutic method for increasing the biomechanical stability of the cornea through the induction of new additional links between collagen fibers. The aim of this paper is to show the mechanism of action of this therapeutically method in different corneal diseases, for which this method can be applied: corneal ectasia, bullous keratopathy, corneal ulcers. The results obtained for two cases with keratoconus and one case with bullous keratopathy are presented as examples.

Key Words: corneal collagen cross-linking, keratoconus, corneal ulcers, bullous keratopathy

Corneal collagen cross linking with riboflavin (C3-R) is currently accepted as a treatment in corneal ectasia after refractive surgery, keratoconus, pellucid marginal degenerescence, bullous keratopathy, corneal dystrophy, corneal ulcers with infectious etiology.

Keratoconus is a relatively frequent disease (1/2000 cases). It is bilateral, with asymmetric evolution, with unknown etiology, and is characterized by central or paracentral conical ectasia of cornea and corneal thinning at cones level. Keratoconus affects the quality of life by reduction of a visual acuity due to the irregular astigmatism induced by loosening the arrangement of collagen fibers, reduction of the number of collagen fiber and of the links between them and imposes the addition to the therapeutic arsenal, besides optical treatment with aerial or contact lenses, other methods more or less invasive: corneal collagen cross linking or the implant of corneal intrastromal rings to delay and diminish the number of patients that require the penetrating keratoplasty procedure.

Bullous kerathopathy is characterized by corneal edema, which may involve all layers, and is usually a sequel of intraocular surgery performed in the presence of certain factors: previously endothelial
disease or lost of endothelial cells, intraoperative endothelial trauma, postoperative inflammation or an increase intraocular prolonged pressure after surgery.

Collagen cross-linking is a naturally occurring or iatrogenically induced reaction that consists in formation of new intermolecular bridges between collagen molecules, resulting in an increased consistency and resistance of the corneal stroma.

The photo-polymerization process starts by oxidative deamination and is induced through the action of UVA radiations on riboflavin that leads to atomic oxygen formation; in its turn, this induces the formation of additional covalent bridges between molecules and it is followed later by repopulation with healthy keratocytes from deep stroma and corneal periphery.

Changes in the intrinsic properties of corneal collagen and the photo-polymerization process are produced in anterior stroma at a depth of 300 μm. Besides the effect of an increased resistance and biomechanical stability of cornea, an increase of a collagen fibers diameter and an anti-collagenase effect is induced, together with stimulation of keratocytes apoptosis.

Besides the absorption and concentration of UV radiation and stimulation of formation of free oxygen radicals, riboflavin has also the role to protect intraocular structures (endothelium, lens, retina). Riboflavin decreases with about 95% the UVA light intensity, allowing it to remain below the endothelium cytotoxic threshold (0.36 mW/cm²) for corneal thickness bigger 400 μ, a dose that is ten times smaller than what would be obtained if riboflavin was not used. In the normal eye, UVA is absorbed mainly by the lens, which also contains endogenous riboflavin and other photosensitizers.

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Regardless of the pathology for which this treatment is made, in principle the therapeutic steps are the same. The intervention is made in aseptic conditions, under topical anesthesia, after the removal of corneal epithelium on an area of 9 mm and instillation of riboflavin 0.1% in Dextran T500 every 3 min for 30 min, followed by UVA - light exposure λ = 370 nm, 3mW/cm² for 30 min, time in which the riboflavin instillation continues every 5 min. Before the radiation initiation it is compulsory to check at slit-lamp the penetration of riboflavin through the cornea in the anterior chamber using a blue filter, to ensure this protective effect.

Postoperatively, therapeutical contact lenses and topical treatment with antibiotics and artificial tears are applied until corneal epithelization ensues, followed by topical administration of steroid anti-inflammatory for two weeks.

For exemplification we present the evolution of two cases of keratoconus and one case of bullous keratopathy.

**Case 1:**

Female patient, 32 years old, with keratoconus stage II at left eye. C3 - R was performed at this eye. Before intervention the following parameters were registered:

- Refraction: -3.25sf <- - 5.50 cyl ax 117;
- Uncorrected visual acuity (UCVA) was 0.05.

At slit – lamp examination we observed corneal ectasia, Vogt’ lines, Fleischer ring. The corneal thickness measurement by ultrasonic pachymetry was 443 μm. Topographic map aspect revealed Kmax = 51.90D ax 15, Kmin = 46.83D ax 110. (Fig. 1)

Three months postoperatively we noticed reduction of the astigmatism with 1.50 diopters, registered refraction was - 3.25 sf <- - 4 cyl ax 115, improvement of the uncorrected visual acuity at 0.1 and corneal thickness 466 μ. Flattening of corneal ectasia on topographic map was: Kmax = 50.12D ax 15, Kmin = 46.06 D ax 110, as shown in Figure 2.

**Figure 1.** Preoperative topographic map for the first case.

**Figure 2.** Postoperative topographic map for the first case.
Case 2:
A 42 years old male patient with advanced keratoconus (stadium III) with the following preoperative parameters:
- Refraction: -10.75 sf <-> -3.25 cyl ax 175;
- Best corrected visual acuity was 0.1 with -11 sf.
Pachymetric corneal thickness was 409 μm and the topographic aspect is: Kmax = 63.48D ax 89, Kmin = 58.93D ax 3. (Fig. 3)

Figure 3. Preoperative topographic map for the second case.

At 6 months postoperatively the values of refraction, visual acuity and corneal thickness are the same, but on the topographic map corneal flattening was noticed: Kmax = 61.23 D ax 0, Kmin = 54.23 D ax 88. (Fig. 4)

Figure 4. Postoperative topographic map for the second case.

Case 3:
A 66 years old female patient with bullous keratopathy in the right eye, in whom cross-linking was performed. Preoperatively the visual acuity at right eye was light perception, intraocular pressure was 40 mmHg (treatment with Timolol and Xalatan), slit-lamp examination showed epithelial and stromal edema, posterior chamber pseudophakia; at ophthalmoscopy no details were noticed due to corneal edema and the corneal thickness could not be recorded. Hypotension treatment is changed by adding topical and systemic anhydrase carbon inhibitor, therapeutic contact lens was applied and C3-R was recommended. One month post-intervention, at slit-lamp examination we noticed the reduction of the corneal edema and discomfort of the patient. Furthermore, an improvement of visual acuity (0.01) was obtained. Figures 5 and 6 present slit-lamp examination before and after intervention.

Figure 5. Preoperative slit-lamp examination for the third case.

Figure 6. Postoperative slit-lamp examination for the third case.

DISCUSSION

Despite extensive research, the etiology of keratoconus has yet to be unraveled, even if its molecular and cellular changes have been established.

Recent studies suggest that the enzymatic anomalies at the level of corneal epithelium, such as the increase in lysosomal enzymes (cathepsin B and G), that act on matrix metaloproteases, and the reduction of the inhibitors of proteolytic enzymes play an important role in the degradation of corneal stroma. Furthermore, the reduction of the activity of α1-protease enzymatic inhibitors is responsible for the stromal gelatinolytic activity. Genetic anomalies of the genes coding these enzymes have been found.

Eye rubbing and contact lens wear may stimulate the release of cytokines (such as interleukin 1) at the epithelial level and the stimulation of keratocyte apoptosis, initially in the anterior stroma, manifested through tears of the Bowman membrane, and subsequently with stromal thinning by defective...
formation of extracellular components, reduction of collagen fibers.

The ocular manifestations of keratoconus include steepening of the cornea, especially inferiorly, thinning of the corneal apex, clearing zones or scarring at the level of Bowman's layer, deep stromal stress lines, ring of iron deposition in the epithelium (Fleischer ring). The symptoms of keratoconus include decreased acuity by myopic or irregular astigmatism, polyopia and decreased contrast sensitivity.

Corneal collagen cross-linking is applied for patients with progressive keratoconus in the last 6 months (a reduction of visual acuity, a decrease in the corneal thickness of more than 20 μ, and the increase in maximal diopter values with at least 1 D), without opacities, with a corneal thickness > 400 μ, and with the number of endothelial cells greater than 2000 cells/mm², without others corneal disease (herpes keratitis).

C3 - R may be applied also in advanced cases with transparent and corneal thickness smaller than 400 μ, but with certain cautions such as using first hypoosmolar riboflavin until an increase of the corneal thickness over 400μ is obtained, measured intraoperatively by ultrasonic pachymetry, after initiation of UVA- light exposure, isoosmolar riboflavin may be used. The effects of this therapy are obtained after several months, not immediately. In literature data, after this procedure an average flattening of corneal ectasia of two diopters is reported, documented topographically, and an increase of corneal thickness with 10-20 μ.2

The improvement of visual acuity was obtained in first case probably by the decrease of corneal curvature or homogenization of the cornea and decrease of the astigmatism. Corneal thickness was modified with 23 μm by increasing the diameter of the collagen fibrils and the hydrophilic capacities of the stromal proteoglycans. Corneal flattening was reduced in both cases with keratoconus, but was obtained after a long time for the second case.

Bullous keratopathy is caused by the perturbation of the activity of Na-K-ATP pump and by the change of NaHCO₃ at the level of endothelial cells which determine an abnormal corneal hydration level.3 At the endothelium level, an increase and deformation of cells, with stromal edema, especially in the central area of cornea is noticed. The edema may fluctuate function of the changing of intraocular pressure, and thus it is extremely important to keep the intraocular pressure at a low level. Epithelial edema is showed by development of epithelial boules due to the accumulation of fluids between epithelial basal cells and is also the result of the anterior movement of fluid in function of intraocular pressure.4

Due to these pathological phenomena these patients present with reduction of the visual acuity, pain, discomfort, photophobia, and epiphora. Epithelial and stromal edema is responsible for changes in visual acuity through irregularity of the refractive corneal surface. The pain is determined by epithelial edema and lesions on nerves fibers.

To diminish the patient discomfort and to delay the procedure of corneal keratoplasty, collagen cross-linking with riboflavin has been introduced in the therapeutic management together with drug therapy for the phak and pseudophak patients (hypotonic, corneal epithelisation, hypertonic agents and hydrophilic soft contact lenses). The use of this method is based on the concept of stromal compaction with the increase of the osmotic and hydrostatic resistance against fluid accumulation of riboflavin under UVA – light action.5 In our case with bullous kerathopathy, a gradual reduction of the corneal edema was observed with subsequent improvement of the corneal clarity and of the corneal epithelium integrity and an improvement of the symptoms without the presence of any side effects.

Recently, corneal collagen cross-linking has been used also in the treatment of the corneal ulceration that does not respond to the medical therapy, with the exception of herpetic corneal ulcers that represent a contraindication. At the level of these pathological lesions bacterial toxins are released, which determine the destruction of glycosaminoglycans although they do not have a direct collagenolytic effect. The ratio between the concentration of tissular collagenases (protease, pepsin, cathepsin) released at the ulcer site, which cleave the collagen fibers, and the activity of the tumor and cellular inhibitors will determine the evolution of the disease.6 Research studies have demonstrated that riboflavin under the action of UVA-light significantly increases the resistance of the keratoocytes from the anterior half of cornea at the action of collagenases, pepsin and trypsin digestion, as riboflavin easily penetrates the corneal stroma when epithelial barrier is interrupted; this effects is also associated with an increase in the biomechanical corneal stability. UVA - light and oxygen radicals have also an antimicrobial effect interfering with the integrity of the microbial wall and retarding the development of bacteria and fungi.6

The use of collagen cross-linking in severe myopia is still in the hypothetical phase. This situation is characterized by a reduction of scleral biomechanical parameters that are the basis for progressive scleral thinning. Radiations with λ = 465 nm are absorbed by
the sclera in a proportion of 35%; 57% are reflected, and 8% of the unreflected radiation is transmitted farther and this is sufficient to produce scleral cross-linking after covering it with riboflavin.57

CONCLUSIONS

Corneal collagen cross-linking is a simple and easy procedure which, by increasing biomechanical corneal stability, through raising the number of bonds between collagen fibers, may stop the evolution of corneal ectasia and also may have a favorable effect in infectious processes or bullous keratopathy. In the future corneal collagen cross-linking might become a standard procedure which will delay and reduce the number of cases that need penetrating keratoplasty as the final solution of corneal disease treatment.

REFERENCES

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