



ANALYSIS OF LONG-TERM RESULTS OF COLLAGEN CORNEAL CROSS-LINKING IN PATIENTS WITH ECTATIC FORMS OF CORNEAL DYSTROPHY

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✧ Corneal collagen cross-linking became a permanent part of complex treatment for patients with different forms of corneal ectasia. In periodical literature, there are anecdotal reports concerning long-term results of this corneal disease therapy method, which is an isolated variant of photodynamic therapy.

Purpose. To carry out a retrospective study of corneal collagen cross-linking long-term results in various ectatic corneal diseases. **Materials and methods.** Results of corneal collagen cross-linking in patients with ectatic forms of corneal dystrophy 6 years after surgery were analyzed. The nosological structure of the study included a group of patients with primary keratoconus, pellucid marginal corneal degeneration, secondary ectasias. The group with primary keratoconus includes 30 patients (31 eyes), that with pellucid marginal degeneration 10 patients (10 eyes), that with secondary ectasias – 10 patients (10 eyes). Data of the diagnostic examination before surgery, intermediate data of the dynamic follow-up during 6 years of observation were used for the analysis. Corneal collagen cross-linking was performed in the first or second year of follow-up, followed by monitoring of changes in the state of the cornea after corneal collagen cross-linking for 4-5 years. **Results.** A statistically significant increase in visual acuity was observed after the corneal collagen cross-linking in patients with primary and secondary ectasias. In patients diagnosed with pellucid marginal degeneration, there was no statistically significant increase of visual function. A decrease in the corneal asymmetry index was revealed in all groups and confirmed by statistical analysis.

✧ **Keywords:** collagen cross-linking; riboflavin; modifications; ultraviolet radiation.

АНАЛИЗ ОТДАЛЁННЫХ РЕЗУЛЬТАТОВ КОЛЛАГЕНОВОГО КРОССЛИНКИНГА РОГОВИЦЫ У ПАЦИЕНТОВ С ЭКТАТИЧЕСКИМИ ФОРМАМИ ДИСТРОФИЙ РОГОВИЦЫ

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✧ **Введение.** Коллагеновый кросслинкинг роговицы прочно вошёл в комплекс лечебных мероприятий, проводимых пациентам с различными формами эктазий роговицы. В литературе встречаются единичные сообщения об отдалённых результатах этого метода лечения патологии роговицы, являющегося частным вариантом фотодинамической терапии. **Цель** — провести ретроспективное исследование отдалённых результатов применения кросслинкинга роговичного коллагена у пациентов

при различных эктатических заболеваниях роговицы. **Материалы и методы.** Проанализированы результаты кросслинкинга роговичного коллагена у пациентов с эктатическими формами дистрофий роговицы через 6 лет после операции. Нозологическая структура исследования включала группу пациентов с первичным кератоконусом, пеллюцидной маргинальной дегенерацией роговицы, вторичными эктазиями. В группу пациентов с первичным кератоконусом включено 30 человек (31 глаз), с пеллюцидной маргинальной дегенерацией — 10 человек (10 глаз), с вторичными эктазиями — 10 человек (10 глаз). Для анализа использовали результаты обследования до операции, промежуточные данные динамического наблюдения в течение 6 лет. Кросслинкинг роговичного коллагена выполняли в первый или второй год наблюдения, затем производили мониторинг изменений состояния роговицы после кросслинкинга роговичного коллагена в течение 4–5 лет. **Результаты.** Выявлено статистически значимое повышение остроты зрения после кросслинкинга роговичного коллагена у пациентов с первичным кератоконусом и вторичными эктазиями. В группе пациентов с диагнозом «пеллюцидная маргинальная дегенерация» статистически значимого повышения зрительных функций не определялось. Выявлено снижение индекса асимметрии роговицы во всех группах, что подтверждает статистический анализ.

✦ **Ключевые слова:** коллагеновый кросслинкинг; рибофлавин; модификации; ультрафиолетовое излучение.

INTRODUCTION

The key pathogenic mechanism underlying the development and progression of ectatic corneal dystrophy involves alterations in the biochemical and biomechanical properties of the corneal collagen lattice. Comparison between biomechanical characteristics of normal and ectatic corneas has demonstrated changes in the structure of collagen in the latter. This observation led researchers to investigate methods for the formation of new cross-links between structural elements of tissues. In polymer chemistry, cross-linking is an established method to increase the elasticity and the strength of materials [13].

Actuality

The development and implementation of novel, more effective, and minimally invasive treatments for corneal disorders (e. g., ectatic corneal dystrophy) remains highly relevant to ophthalmologists [1]. Corneal ectatic disorders are classified into primary and secondary ectasias. Primary corneal ectasias include eye conditions such as keratoconus, keratoglobus, and pellucid marginal degeneration (PMD, keratotorus). Secondary (iatrogenic) ectasias are induced by laser refractive surgeries, such as laser keratomileusis, photorefractive keratectomy, phototherapeutic keratectomy [19], anterior radial keratotomy, and corneal transplantation.

A group of non-inflammatory peripheral corneal thinning disorders has been associated with age-related changes or systemic diseases. This group includes Terrien marginal degeneration and marginal

thinning associated with drying or systemic diseases, such as scleroderma, rheumatoid arthritis, and Wegener granulomatosis.

The reported prevalence of corneal ectatic disorders in the general population is 1 per 2,000. These disorders are most commonly diagnosed in young individuals. Approximately 20% of patients experience rapid progression of the disease and require penetrating keratoplasty. Recently, the scope of photodynamic therapy has been expanded, especially in ophthalmology. In 2003, Seiler et al. developed and implemented the technique of corneal collagen cross-linking (CXL) using riboflavin as a photosensitizer that initiates the photochemical modification of monochromatic ultraviolet light. CXL is currently recognized as the only effective treatment capable of slowing down the progression of keratoconus by improving the biomechanical properties of the cornea [20, 21]. Modified CXL protocols reduce the operative time without impairing the biomechanical effects in the cornea (wavelength: 365 nm; power-flux density: 9 mW/cm²; exposure time: 10 min) [3].

Corneal biomechanics

Biomechanical properties of the cornea are primarily associated with the stroma, which constitutes approximately 90% of the total corneal thickness. The main components of the stroma are collagen macromolecules forming long fibrils of equal diameter (31–34 nm), which are further assembled into stabilized fibers called lamellae (1–2 μm in thickness and 100–200 μm in width). The specific firmness of fibrils is the result of physiologi-

cal cross-linking between collagen molecules. CXL is enzymatically regulated by lysyl oxidase, which catalyzes the amino groups of amino acids (e. g., lysine) into aldehyde groups. Subsequently, these aldehyde groups form covalent bridges between each other or with other amino acids within one or different fibrils. Patients with keratoconus were found to have defects in the gene encoding lysyl oxidase. Moreover, a high pH in tears alters the activity of lysyl oxidase. Studies have demonstrated that a decrease in the levels of protease inhibitors leads to higher enzymatic activity [5, 7, 9].

The spatial arrangement of the corneal collagen fibers is another important factor affecting the biomechanical properties and the shape of the cornea. The elastic modulus is proportional to the branching density of collagen fibers. Therefore, biomechanical properties depend on the interconnectivity of collagen fibers. This characteristic is most important in the third corneal layer (stroma), making it the target of CXL [16, 19].

Biochemical mechanism of CXL efficacy

The aim of CXL is to accelerate the physiological mechanism of protein glycation. CXL creates bonds between collagen molecules to strengthen the anterior corneal stroma. The monochromatic ultraviolet light acts as a catalyzer. Riboflavin (vitamin B2) acts as photosensitizer absorbing photon energy and is subsequently activated, thereby creating covalent bonds between amino groups within the collagen molecules or between proteoglycans and collagen [16]. A long-term photochemical reaction involving riboflavin and low-intensity ultraviolet light consolidates collagen α and β chains into larger polymers. In addition, it crosslinks major proteoglycans (e. g., keratocan, lumican, mimecan, and decorin) into high molecular weight polymers. Moreover, crosslinks are formed between collagen and proteoglycan core proteins. This reaction requires oxygen and generates oxygen-free radicals through photolysis of riboflavin [18].

Another potential *ex vivo* mechanism of CXL is an increase in the production of fibronectin and tissue transglutaminase – an enzyme cross-linking the proteins of the extracellular matrix – in human keratocytes treated with CXL [22].

Numerous clinical trials have demonstrated the efficacy and safety of CXL in the treatment of progressive keratoconus. *Ex vivo* studies have demonstrated that CXL increased elasticity without reducing corneal transparency [11]. Of note, resistance to enzymatic degradation was also in-

creased. However, it is currently not possible to demonstrate the sustainability of these effects because of the physiological renewal of corneal collagen molecules every 2–3 years. Therefore, it remains unclear whether the changes observed in corneal stability are permanent or temporary. Furthermore, the selection of the most appropriate methods to maintain the positive effects remains unclear [15]. Studies with longer follow-up periods should thoroughly review the effects of the standard and modified CXL protocols. In an uncontrolled retrospective study involving a large cohort of patients with keratoconus followed up for 6 years, Raiskup-Wolf et al. demonstrated that corneal flattening was sustained for several years and remained stable [14]. A number of studies demonstrated that progression of keratoconus was arrested in 90% of cases. In addition, significant improvement in the best-corrected visual acuity was observed [8, 20].

The aim of the present study was to analyze the long-term outcomes of CXL in patients with various corneal ectatic disorders.

MATERIALS AND METHODS

We followed up patients with various corneal ectatic disorders for 6 years after treatment with CXL. The study cohort included 50 patients (51 eyes) aged between 13 and 45 years (mean age: 26.53 ± 7.69 years). All patients were divided into three groups according to the type of ectatic disorder: primary keratoconus (30 patients, 31 eyes), PMD (10 patients, 10 eyes), and secondary keratectasias (10 patients, 10 eyes).

We measured the best-corrected visual acuity at the first (prior to CXL) and last examinations (after CXL). In addition, we determined the surface asymmetry index (SAI) – the most reliable indicator of ectatic processes in the cornea – prior to CXL and during the 6 year follow-up period.

All patients underwent comprehensive examination that included biomicroscopy, ophthalmoscopy, ophthalmometry, refractometry, visual acuity testing, tonometry, and perimetry. In our analysis, we used the results obtained at the first and last examinations. CXL was most commonly performed during the first or second year of follow-up. Subsequently, the patients were monitored for corneal changes during the remaining 4–5 years of follow-up. Particular attention was paid to keratotopography using the Tomey TMS-4 Corneal Topographer. CXL was performed using the UV-X 1000 Illumination

System (wavelength: 365 nm; power-flux density: 3 mW/cm²; intensity: 3 mW/cm) with 0.1% riboflavin solution (Dextralink).

RESULTS

In patients with primary keratoconus and secondary ectasia, a statistically significant increase in visual acuity was observed after CXL. Patients with PMD did not exhibit significant changes in their visual function, which may be attributed to specific characteristics of the disease (Table 1).

A significant decrease in SAI was reported in all groups. Analysis of the long-term outcomes of CXL demonstrated the high efficacy of the procedure, a finding consistent with that of Raiskup-Wolf et al. [14]. In addition, a negative correlation between SAI and best-corrected visual acuity was found in all groups (Figs. 1–3).

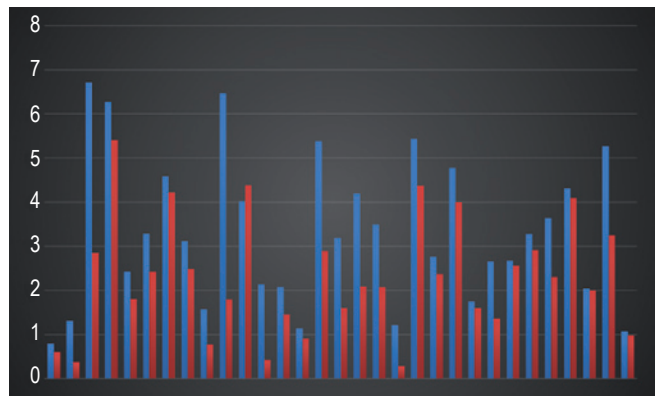


Fig. 1. The value of the corneal asymmetry index before and after CXL with primary keratoconus

Рис. 1. Значение индекса асимметрии роговицы до и после кросслинкинга роговичного коллагена при первичном кератоконусе

Table 1
Comparison of best corrected visual acuity and corneal asymmetry index in patients with ectatic forms of corneal dystrophy before and after collagen corneal cross-linking

Таблица 1
Сравнение максимальной корригированной остроты зрения и индекса асимметрии роговицы у пациентов с эктатическими формами дистрофий роговицы до и после коллагенового кросслинкинга роговицы

Disorder	Parameter			
	Best-corrected visual acuity prior to CXL	Best-corrected visual acuity after CXL	Surface asymmetry index prior to CXL	Surface asymmetry index after CXL
Primary keratoconus	0.56 ± 0.29	0.79 ± 0.28	3.32 ± 1.68	2.27 ± 1.33
Pellucid marginal degeneration	0.54 ± 0.25	0.51 ± 0.32	1.89 ± 0.96	1.79 ± 0.81
Secondary ectasias	0.5 ± 0.26	0.74 ± 0.31	6.44 ± 1.29	3.63 ± 1.23

Note: CXL, corneal collagen cross-linking

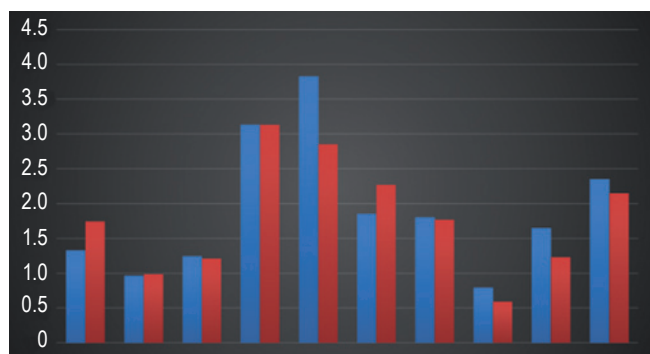


Fig. 2. The value of the corneal asymmetry index before and after CXL with pellucid marginal degeneration of the cornea

Рис. 2. Значение индекса асимметрии роговицы до и после кросслинкинга роговичного коллагена при пеллюцидной маргинальной дистрофии роговицы

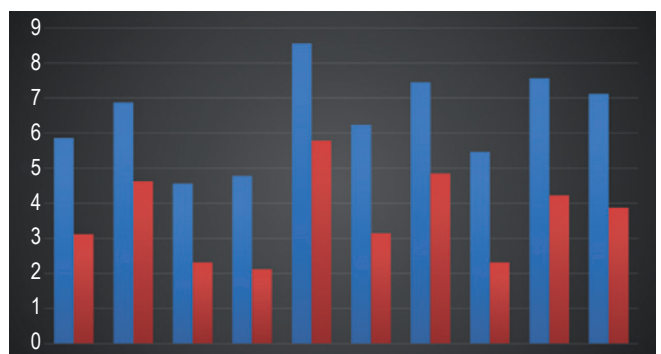


Fig. 3. The value of the corneal asymmetry index before and after CXL with secondary keratoconus

Рис. 3. Значение индекса асимметрии роговицы до и после кросслинкинга роговичного коллагена при вторичном кератоконусе

DISCUSSION

The use of photodynamic therapy and its implementation into clinical practice has led to advances in the treatment of complex corneal pathology. Our understanding of the photochemical processes responsible for alterations in the biomechanical properties of the cornea has expanded the indications of treatment with CXL. Thus far, multiple modifications in the protocol of the CXL procedure have been reported and researchers continue to investigate new CXL regimens and photosensitizers. Recently published studies demonstrated that the use of longer-wavelength light is favorable in terms of the development of mutations and carcinogenesis. Studies have also suggested using other agents capable of initiating photochemical polymerization and cross-linking of corneal collagen fibers. Considering that these modifications in the CXL procedure have only been developed recently, data on the long-term outcomes of this treatment are currently limited.

The present study demonstrated positive effects of treatment with CXL in patients with primary keratoconus and secondary corneal ectasia, including arrested progression of keratoconus and complete stabilization of the pathological process. In some cases, significant changes in keratotopography parameters and corneal optical power were observed, resulting in significant improvement of visual functions and effective use of commercially available products for vision correction. A proportion of patients may achieve complete rehabilitation in their professional activity and report improvement in their quality of life. However, several patients with PMD failed to achieve significant improvement in their best-corrected visual acuity. In recent years, the CXL protocol has undergone significant alterations primarily aimed at accelerating the procedure, increasing the radiation power, and changing the exposure parameters without modifying the dosage. CXL increases visual acuity in patients with corneal ectatic disorders, encouraging further improvement of the methodology and determination of the optimal time for its use.

CONCLUSIONS

CXL is an effective procedure capable of slowing down the progression of keratoconus and stabilizing various corneal ectatic disorders. Moreover, CXL increases visual acuity, thereby improving patient's quality of life.

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The authors do not have conflicts of interest to declare.

Author contribution: S.Yu. Astakhov and S.A. Novikov developed the research concept and study design; O.A. Frolov and P.A. Danilov performed the data collection, processing, and analysis, and drafted the manuscript.

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