Prophylactic and Therapeutic Laser-Induced Corneal Crosslinking

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Abstract

Purpose: To consider new approaches to prophylactic and therapeutic corneal crosslinking based on the activation of riboflavin by induced secondary radiation of an argon-fluorine excimer laser.

Material and Methods: The work is based on 7 years of experimental (90 rabbit eyes) and clinical studies of the use of riboflavin for photoprotection and laser-induced crosslinking in photorefractive (498 operations) and phototherapeutic (66 operations) corneal surgery. In all cases, stromal saturation with 0.1% or 0.25% isotonic riboflavin solution was performed after phototherapeutic ablation of the epithelium. The choice of riboflavin concentration and the duration of saturation of the stroma with riboflavin depended on the volume of tissue removed, the degree of ametropia, and the type of corneal pathology. In experimental studies, biomechanical testing, light and transmission electron microscopy were carried out. In the clinic, corneal crosslinking was assessed by corneal topography, optical coherence tomography, and densitometry.

Results: Experimental studies revealed the effect of laser-induced crosslinking when exposed to different energy densities of an argon-fluorine excimer laser. For prophylactic crosslinking in photorefractive and phototherapeutic corneal surgery, riboflavin activation by secondary radiation induced during ablation was sufficient. For therapeutic crosslinking, only the radiation energy densities of an argon-fluorine excimer laser below the ablation threshold were used. A special technology using subablative energy densities has been applied to form a Bowman-like membrane structure on the ablative surface. Therapeutic laser-induced crosslinking has shown its effectiveness in keratoconus, secondary keratoectasias, infectious keratitis, and other corneal pathologies. Scanning the cornea with a narrow beam of an ophthalmic excimer laser using argon-fluorine made it possible to carry out personalized therapeutic corneal crosslinking according to the data of corneal topography. Following therapeutic laser-induced crosslinking, all the classic signs of crosslinking with the formation of a demarcation line were revealed on corneal OCT.

Conclusion: Prophylactic or therapeutic laser-induced corneal crosslinking can be realized through the activation of riboflavin by secondary radiation induced at ablative or subablative energy densities in the radiation pulse of an argon-fluorine excimer laser.

Keywords: Laser-Induced Corneal Crosslinking; Argon-Fluorine Excimer Laser; Photorefractive Keratectomy; Phototherapeutic Keratectomy; Keratoconus; Riboflavin

Abbreviations

PRK: Photorefractive Keratectomy; TransPRK: Transepithelial Photorefractive Keratectomy; PTK: Phototherapeutic Keratectomy; Femto-LASIK: Femtolaser In Situ Keratomileusis; CXL: Collagen Cross-Linking; LASIK Xtra: Laser In Situ Keratomileusis + Collagen Cross-Linking; FemtoLASIK Xtra: FemtoLaser In Situ Keratomileusis + Collagen Cross-Linking; KCN: Keratoconus; UV: Ultraviolet; UV-A: Ultraviolet-A; UV-B: Ultraviolet-B; D: Diopter; OCT: Optical Coherent Tomography

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Introduction

The arsenal of different technologies for corneal crosslinking is expanding every year [1-5]. Initially, the traditional Dresden Protocol medical crosslinking technology was developed for the treatment of keratoconus and secondary keratoectasias of various etiologies. Subsequently, various modifications of this technology appeared for the prevention of keratoectasias in laser refractive and optical-reconstructive surgery of the cornea. Prophylactic corneal crosslinking could be considered as a way to compensate or reduce the weakening of the biomechanical and photoprotective properties of the cornea during its thinning. The strength properties of the cornea were most significantly impaired during photorefractive operations. This concerned, in particular, technologies of preventive accelerated corneal crosslinking during operations of excimer laser in situ keratomileusis with mechanical (LASIK Xtra) or femtolaser (FemtoLASIK Xtra) formation of a superficial flap. When applying these technologies, the time of crosslinking was reduced by increasing the UV flux density while maintaining the total dose of 5.4 J/cm² [3-5]. However, the combination of photorefractive or phototherapeutic operations with additional UV radiation increased oxidative stress in the cornea and increased the risk of complications [6-8]. In addition, with traditional or accelerated crosslinking technologies, the cornea became excessively rigid and its ability to smooth out accommodative-convergent IOP fluctuations was impaired. That is why, until now, the advisability of using corneal crosslinking for prophylactic purposes is being debated among laser refractive surgeons.

Purpose of the Study

To consider new approaches to prophylactic and therapeutic corneal crosslinking based on the activation of riboflavin by induced secondary radiation of an argon-fluorine excimer laser.

Material and Methods

The work is based on 7 years of experimental (90 rabbit eyes) and clinical studies on the use of riboflavin for photoprotection and laser-induced crosslinking in photorefractive (498 operations) and phototherapeutic (66 operations) corneal surgery. For prophylactic crosslinking during photorefractive surgeries, only secondary radiation induced during ablation was used. In some cases, the crosslinking effect was enhanced by additional exposure to energy densities below the ablation threshold. A special technology using subablative energy densities has been applied to form a Bowman-like membrane structure on the ablative surface. The saturation of the stroma with 0.1% or 0.25% isotonic solution of riboflavin was carried out by drip or aerosol method. In the aerosol method of saturation, a portable ultrasonic nebulizer with mesh technology for solution dispersion was used. With this technology, the size of aerosol particles of the riboflavin solution ranged from 0.5 to 4 microns. This improved their absorption on the de-epithelialized surface of the stroma. In all cases, during prophylactic crosslinking in photorefractive surgery, the epithelium was removed in the PTK mode, taking into account its thickness according to the OCT of the cornea. For therapeutic purposes, subablative energy densities have been used for crosslinking in keratoconus, secondary corneal ectasias, infectious keratitis, and other corneal pathologies. All studies were carried out in compliance with the principles of the Declaration of Helsinki and with the permission of the Ethics Committee of the FSBI "National Medical and Surgical Center named after NI Pirogov" of the Ministry of Health of Russia. In experimental studies, biomechanical testing, light and transmission electron microscopy of corneal samples after ablation, without and with preliminary saturation with riboflavin, were carried out. Photorefractive and phototherapeutic surgeries using excimer laser radiation below the ablation threshold were performed using the Russian excimer laser Microscan Visum-500. In this laser system, the ability to quickly switch from ablative to subablative modes was realized, without any additional control calibrations. In the clinic, when assessing the state of the cornea after excimer laser crosslinking, special emphasis was placed on high-resolution spectral optical coherence tomography (OCT) and corneal densitometry. The studies were carried out on RTVue 100 and RTVue XR100 devices (Optovue, USA). Keratotopographic and densitometric studies were performed using a TMS-5 device (Topcon, Japan).
Results and Discussion

Experimental studies of rabbit eyes have revealed a decreased aseptic inflammatory response, accelerated epithelialization and an average increase of strength properties by 45%. In tested cornea samples after photoablation with riboflavin. Thus, in in vivo experiments the tensile strength of corneal samples after ablation to a depth of 100 μm preceded by stroma saturation with 0.25% isotonic riboflavin solution was 12.8 ± 1.3 MPa. With a similar ablation without riboflavin, the tensile strength was significantly (p = 0.0004) lower and amounted to 8.8 ± 0.9 MPa. Keratopachymetric studies, as well as data from optical coherence tomography of the cornea, showed that saturation of the corneal stroma with 0.1% or 0.25% isotonic riboflavin solution does not affect the accuracy of ablation. According to transmission electron microscopy, after photorefractive keratoablation with riboflavin, the formation of stable cross-linking of collagen fibers was observed in the layers of the cornea adjacent to the ablation zone. When examining cross-sections of collagen bundles in ablated corneas with riboflavin, an increase in the number of cross-links and compactness of collagen fiber packing was revealed (Figure 1, arrows). This was indicated by a halving of the distance between fibrils per unit area. With keratoablation without riboflavin, such changes were absent. Experimental studies have shown that secondary radiation induced at different energy densities of an argon-fluorine excimer laser can be used for corneal crosslinking. The results of these studies were presented in more detail in previously published works [9-12]. Clinical observations have revealed the advantage of photorefractive ablation with preliminary saturation of the stroma with riboflavin. This manifested as a decrease in the aseptic inflammatory response in the early postoperative period (Figure 2), acceleration of epithelialization in TransPRK, and a reduction in the time required for the stabilization of visual and optical refractive indices. Analysis of the results of various photorefractive and phototherapeutic operations on the cornea confirmed the data of experimental studies that stromal saturation with 0.1% or 0.25% isotonic riboflavin solution has no effect on the accuracy of ablation. At the same time, riboflavin-saturated layers of the corneal stroma acted like spectral filters, protecting keratocytes and nerves in deeper layers of the stroma from the negative effects of ablation-induced secondary UV radiation on them. The technology of corneal photoablation with riboflavin was protected by a patent for invention [13] and its results are discussed in more detail in previously published works [16-18].

Figure 1: Ultrastructural changes with the formation of stable cross-linking of collagen fibers in the stroma of the rabbit cornea after laser-induced crosslinking. The compactness of the fiber packing is noted due to the increase in the number of cross-links (arrows). Transmission electron microscopy, magnification x 25000.
Experimental and clinical studies of photoablation with riboflavin formed the basis for the development of laser-induced corneal crosslinking in the clinic. The technology of such crosslinking consisted in the use of secondary radiation to activate riboflavin. This radiation was formed when the stroma was exposed to radiation from an argon-fluorine excimer laser at ablative and subablative pulse energy densities. The broad spectrum of induced secondary radiation included the ultraviolet range, which overlapped all four peaks of maximum absorption by riboflavin [17-21]. This indicated the advantages of using secondary radiation from an argon-fluorine excimer laser to initiate the crosslinking effect in the corneal stroma. Clinical observations have shown that in most cases of photorefractive surgery of the cornea for the prevention of keratoectasias, it is sufficient to carry out ablation after saturation of the corneal stroma with 0.1% or 0.25% isotonic riboflavin solution. Depending on the degree of ametropia and the volume of tissue removed, the saturation time ranged from 2 to 10 minutes. Riboflavin provided photoprotection of deeper stromal structures from the negative effects of ablation-induced secondary radiation. Simultaneously, upon completion of ablation, the crosslinking effect was initiated in the adjacent layers of the stroma. In this case, a thin membrane structure was formed on the ablative surface [22]. During OCT of the cornea, the membrane was detected only in cases when its thickness exceeded 5 μm. This was due to the resolution used by the optical tomograph. With corneal densitometry, an increase in optical density was observed in the stromal layers adjacent to the ablation zone. Such laser-induced crosslinking in photorefractive corneal surgery had a prophylactic focus and was more physiological. This was primarily due to the fact that only secondary radiation induced during refractive excimer laser keratectomy was used to activate riboflavin.

In the case of subclinical keratoconus, the effect of laser-induced cross-linking was enhanced by exposure to energy densities below the ablation threshold. To achieve this, immediately after the completion of photoablation with riboflavin, additional irradiation was performed at energy densities below the ablation threshold. In subsequent publications, we will review in more detail the results of using subablative modes of radiation from an excimer laser with a wavelength of 193 nm to modify the ablative surface and form a Bowman-like membrane structure on it.
The technology of therapeutic laser-induced crosslinking is based on saturation of the corneal stroma with riboflavin and the use of pulsed radiation of an argon fluorine excimer laser for its activation at pulse energy densities below the ablation threshold [23]. The use of a small-diameter scanning spot allowed for personalized topographically oriented corneal crosslinking. With scanning technology of crosslinking with a flying spot, oxygenation of the corneal stroma was less disturbed. In addition, a broad spectrum of induced secondary UV radiation increased riboflavin activation, the total extinction coefficient, and corneal crosslinking. This triggered a whole cascade of reactions of active radicals formation. The postoperative aseptic inflammatory response was involved in the formation of such radicals. The nature and severity of this reaction largely predetermined the time of appearance, reverse development, and the severity of the demarcation line in the corneal stroma. Thus, with OCT of the cornea, a deepening of the crosslinking zone in the stroma was noted on the 6th day after laser-induced crosslinking (Figure 3). In all cases, the shape and depth of the demarcation line in the stroma served as a marker of specific corneal zones in which crosslinking occurred. It should be noted that the appearance of a demarcation line was rare in the case of a prophylactic version of crosslinking. The stabilization of the process and the achieved therapeutic effect were judged by differential keratotopography data and the dynamics of the screening ectasia index (Figure 4). In a number of cases, the effect of laser-induced crosslinking was enhanced by using a special technology of applying subablative energy densities to form a Bowman-like membrane structure on the ablative surface. Therapeutic laser-induced corneal crosslinking has shown its effectiveness in endothelial-epithelial corneal dystrophy with severe pain syndrome, infectious ulcerative keratitis and recurrent epithelial corneal erosions (Figure 5-7). In a number of cases with corneal pathology, therapeutic corneal crosslinking was combined with phototherapeutic keratectomy of the opaque or necrotically altered layers of the corneal stroma. The stabilizing result of laser-induced corneal crosslinking was followed up to 1.5 years. The features of the technology for conducting therapeutic laser-induced corneal crosslinking itself will be considered in subsequent publications after its patenting.

**Figure 3:** The state of the anterior segment of the eye and OCT of the cornea of the right eye 1 and 6 days after topographically oriented laser-induced crosslinking for progressive keratoconus grade II. Uncorrected visual acuity increased from 0.1 to 0.6.
**Figure 4**: Differential topographic map 15 months after topographically oriented laser-induced crosslinking for grade II progressive keratoconus. The ectasia screening index decreased by 30%.

**Figure 5**: Dynamics of corneal OCT after laser-induced crosslinking for grade II progressive keratoconus.
Analysis of studies on photoprotection of the intraocular structures of the eye showed that the cornea performs an important function of absorbing medium ultraviolet (UVB) radiation and weakening the intensity of the near (UVA) ultraviolet radiation flux [25,26]. Due to physiological crosslinking, the photoprotective function of the cornea increases with age. This is due to an increase in the number of cross-links in the collagen structures of the corneal stroma. That is why today, therapeutic and prophylactic corneal crosslinking should be considered from the standpoint of increasing the photoprotective function of the cornea. This justifies the expansion of indications for prophylactic laser-induced crosslinking in photorefractive corneal surgery based on photoablation with riboflavin saturation of the

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stroma. According to the Bouguer - Lambert - Beer law, it follows that the thicker the layer of the absorbing medium, the greater the absorption of radiation. That is why, when the cornea becomes thinner, its protective function from external UV radiation is disturbed and the UV load on the lens increases. Our clinical observations have shown that the cataractogenic effect of a particular photorefractive operation depends on a combination of a number of unfavorable factors. Among these factors in terms of durability and duration of adverse effects on the lens, the leading role is played by corneal thinning in the central optical zone [25,26].

Experimental and clinical studies have made it possible to propose a number of innovative technologies for excimer laser corneal surgery with photoprotection, prophylactic and therapeutic corneal crosslinking, which will be reviewed in subsequent publications.

Conclusion

1. For prophylactic laser-induced crosslinking in photorefractive and phototherapeutic operations on the cornea, it is sufficient to saturate the stroma with 0.1% or 0.25% isotonic riboflavin solution and activate it by secondary radiation induced during ablation.

2. Subablative modes of radiation of an argon-fluorine excimer laser can be used to form a Bowman-like membrane structure on an ablation surface.

3. For therapeutic laser-induced corneal crosslinking, it is sufficient to remove the corneal epithelium, saturate the stroma with riboflavin and irradiate it with energy densities in a pulse below the stromal ablation threshold with a total dose of at least 5.4 J/cm².

4. Prophylactic or therapeutic laser-induced corneal crosslinking can be realized through the activation of riboflavin by secondary radiation induced at ablative and subablative radiation energy densities of an argon-fluorine excimer laser.

Bibliography


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