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An Experimental Evaluation of a Photoprotection By Riboflavin in The Excimer Laser Refractive Keratectomy.

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ABSTRACT

To conduct a pilot assessment of the photoprotective effect of Riboflavin in the transepithelial technology of a photorefractive keratectomy.
Keywords: photoprotection, Riboflavin, transepithelial photorefractive keratectomy

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INTRODUCTION

Regardless of the etiological factor, any tissue damage is accompanied by the development of a local oxidative stress and inflammatory response and a regenerative response [1,2].

A refractive surgery on the cornea using laser radiation of different spectral ranges provides the conditions for the development of oxidative stress. The first step was to carry out of photorefractive keratectomy. Thus, during the laser ablation with a radiation of an excimer laser with a wavelength of 193 nm, despite the fact that most of the radiation is absorbed, part of it transforms into the longer waves [3-5]. This leads to a cascading fluorescence of collagen proteins of the corneal stroma and creates an effect of a suggestive secondary fluorescence. Secondary radiation penetrates much deeper than the evaporated layer of the corneal stroma, which leads to excessive accumulation of peroxide radicals in the stroma of the cornea. [6]. Oxide radicals cause damage to the keratocytes, collagen and nerve structures adjacent to the zone of ablation. This affects the severity of postoperative inflammatory reactions and may be accompanied by a regression of the refractive effect and the violation of transparency of the cornea. That's why there is a need in a photoprotection of the cornea [7]. Our attention was drawn to the Riboflavin, which is able to absorb UV radiation and its anti-oxidant properties [8]. A photoprotective effect of Riboflavin on the corneal endothelium was convincingly proven during a crosslinking [9], however, it was not used with the photoprotective aim in the refractive excimer laser ablation of the cornea.

One of the features of the oxidative stress in the cornea during the excimer laser refractive ablation is that the cornea is exposed to a UV load. This load increases with the increase in the volume of a tissue to be removed, which leads to an excessive accumulation of peroxide radicals in the stroma of the cornea. Oxide radicals cause damage to the keratocytes, collagen and nerve structures adjacent to the zone of ablation. This affects the severity of postoperative inflammatory reactions, and may be accompanied by the regression of the refractive effect and the violation of transparency of the cornea [3-11].

GOAL

To conduct a pilot assessment of the photoprotective effect of Riboflavin with the transepithelial photorefractive keratectomy.

MATERIAL AND METHODS

The experiments were performed on 24 eyes of 12 adult rabbits. The transepithelial technology of a photorefractive keratectomy with Riboflavin photoprotection and without it, was carried out consistently on the eyes of the same rabbit. The correlation applied was between sph -7,0 and sph -3,5 diopters with the depth of ablation at the center of 100 µm and 50 µm. After laser de-epithelization, saturation of the stroma was performed and was carried out using a saturation with a spray of 0.25% isotonic solution of Riboflavin for 3 minutes with an ultrasonic nebulizer B. Well WN114. Operations were performed on the excimer laser ophthalmic device Wavelight Allegretto 200. Before and after an operation, the optical coherence tomography of the cornea was performed on the device RTVue-100.

RESULTS

The photoprotective effect of Riboflavin during the transepithelial photorefractive keratectomy was noted in all cases. The degree of the pericorneal injection and the edema of the corneal stroma did not exceed 1 point in the evaluation on a three-point scale. These indicators during the ablation without Riboflavin ranged from 2 to 3 points. In the correction of sph -7,0 diopters and a depth of ablation at the center of 100 µm on the third day after the transepithelial photorefractive keratectomy with Riboflavin, the total score was 6.7 ± 1.4 points, vs 9.8 ± 4.6 points on the pair of eyes without Riboflavin (P=0.037). The difference persisted despite the reduction in a total point scoring within 21 days of the observation. In the transepithelial photorefractive keratectomy without Riboflavin the term of epithelization of the ablation zone was increased.

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CONCLUSION

Photoprotection with Riboflavin in the transepithelial photorefractive keratectomy reduced the severity of a corneal syndrome, accelerated the onset of a complete epithelialization and did not affect the accuracy of the refractive ablation.

MATERIALS AND METHODS

All animal-handling procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and followed the guidelines of the Animal Welfare Act. All animal experiments were approved by the Ethical Committee of Postgraduate Medical Institute "National Pirogov Medical Surgical Centre", Ministry of Health, Moscow, Russian Federation. The animals were housed in individual cages suspended above ground with corncob bedding. All animals were housed throughout the acclimation period and, during the study, in an environmentally controlled room. The control room temperature was set to maintain environmental conditions of 19 ± 3°C. The animal house was kept on a 12 h light/dark cycle. Transepithelial photorefractive keratectomy was performed on 24 eyes of 12 adult rabbits. Transepithelial photorefractive keratectomy with the Riboflavin photoprotection and without it was carried out on the eyes of the same rabbits. After laser de-epithelization, the aerosol saturation of the stroma with 0.25% isotonic Riboflavin solution was carried out for 3 minutes. The solution was prepared ex tempore by diluting 1.0 ml of 1% solution of Riboflavin with 3 ml BSS solution. For the aerosol saturation a nebulizer B. Well WN114 (UK) was used with a membrane-mesh technology of a solution dispersion. In the first series of experiments on 12 eyes was conducted with a correction sph -7,0 diopters and the depth of ablation at the center of 100 microns. In the second series of experiments on the 12 eyes, a correction reached sph -3,5 diopters and the ablation depth at the center was 50 microns. The transepithelial photorefractive keratectomy was performed without Riboflavin, on the same rabbit with the pair of control eyes. Operations were performed with the excimer laser ophthalmic device Wavelight Allegretto 200 (Alcon, USA). Before and after ablation, the optical coherence tomography of the cornea was performed with the device RTVue-100 (Optovue, USA). During the biomicroscopy of the anterior segment of the eye, particular attention was paid to the condition of the cornea. For this reason, we used a specially designed point scoring system for a number of such symptoms such as tearing, photophobia, discharge, pericorneal injection, the area of erosion on the cornea and the character of the epithelial edge, the state of the epithelium upon completion of epithelialization, stromal edema, opacity of the cornea, etc. The severity of each symptom was assessed on a 3-point scale: 1 - light, 2 –mild, 3 –severe. Based on the summation of all of the symptoms, the overall rating was performed, that was placed in the appropriate table of a dynamic observation. The total score ranged from 0 to 27 points. A value of 0 corresponds to the normal state or the absence of the symptom. The postoperative observation period was 21 days. For the reasons, no drugs were used for clarity in the postoperative period.

RESULTS

The photoprotective effect of Riboflavin in the transepithelial photorefractive keratectomy was noted in both series of experiments, but was more significant in the first series. The degree of a pericorneal injection and the edema of the corneal stroma did not exceed 1 point in the evaluation on a three-point scale. These indicators in the ablation without Riboflavin ranged from 2 to 3 points. On the third day after transepithelial photorefractive keratectomy with Riboflavin, the total score was 6.7 ± 1.4 points, vs 9.8 ± 4.6 points on the pair of eyes without Riboflavin ($P<0.001$). This difference persisted despite the reduction in a total score during the whole observation period (tab.1, Fig.1). At the transepithelial photorefractive keratectomy without Riboflavin photoprotection lengthened the term of epithelization of the ablation zones in an average of 2.14 ± 0.46 days.

In the second series of experiments at the 3rd and the 7th days there was no difference in a severity of a corneal syndrome, residual area of de-epithelization and the edema of stroma. This can be explained by the small ablation volume and a weak response with the aseptic inflammatory reaction (see Fig. 2, table 2). However, ($P<0.005$) was on the 14th day and on the 21st day.

Table 1. The score condition of eyes of rabbits after transepithelial photorefractive keratectomy without and with saturation of the corneal stroma by the Riboflavin solution (M ± σ)

Indicators	Period after surgery				
	1 day	3 day	7 day	14 day	21 day
Ablation to a depth of 100 µm with the saturation of Riboflavin (n = 6)	12±1,1	6,7 ± 1,4	4,3 ± 3,9	3,2 ± 3,9	3,8 ± 4,8
Ablation to a depth of 100 µm without saturation of Riboflavin (n = 6)	12,3± 0,5	9,8 ± 4,6	7,5 ± 2,6	6,3 ± 2,6	6,3 ± 3,3
Ablation to a depth of 50 µm with the saturation of Riboflavin (n = 6)	12,6± 0,5	6,2 ± 0,5	1,7 ± 0,5	1,2 ± 0,4	0,8 ± 0,4
Ablation to a depth of 50 µm without saturation with Riboflavin (n = 6)	12,7± 0,5	6,4 ± 0,6	1,7 ± 0,5	2,0 ± 0,1	1,9 ± 0,1

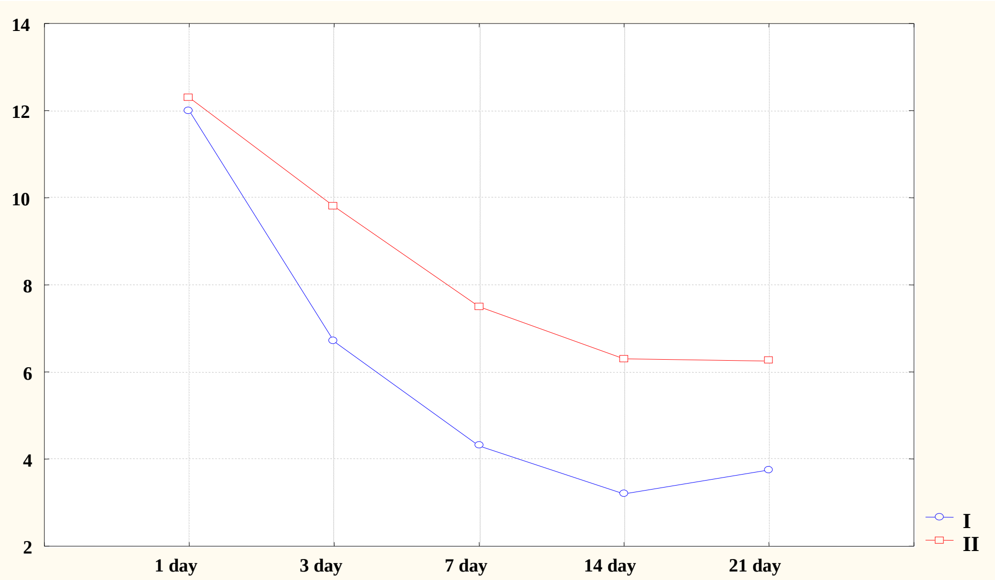

Fig. 1. The total score condition of the eyes of rabbits after transepithelial photorefractive keratectomy with saturation of the corneal stroma by the Riboflavin solution (I) and without (II), with correction sph (-) of 7.0 diopters, and a depth of ablation at the center of 100 microns.

(P<0.001) after transepithelial photorefractive keratectomy with Riboflavin was marked the better state of the epithelium and transparency of the cornea, in comparison with the pair of eyes where the transepithelial photorefractive keratectomy was performed without Riboflavin.

Table 2. The effect of saturation of the cornea with Riboflavin on the accuracy of the ablation in transepithelial photorefractive keratectomy in vivo experiments (M ± σ).

Indicators	The thickness of the cornea before ablation (µm)	The thickness of the cornea after ablation without Riboflavin saturation (µm)	Corneal thickness after ablation with Riboflavin saturation (µm)
Transepithelial photorefractive keratectomy Sph (-) 7,0 diopters, the depth of ablation at the center is 100 microns	322 ±50	239 ±51	224±41
		p=0,6	
Transepithelial photorefractive keratectomy Sph (-) 3,5 diopters, the depth of ablation at the center is 50 microns	348±19	300±20	296 ±21
		p=0,7	

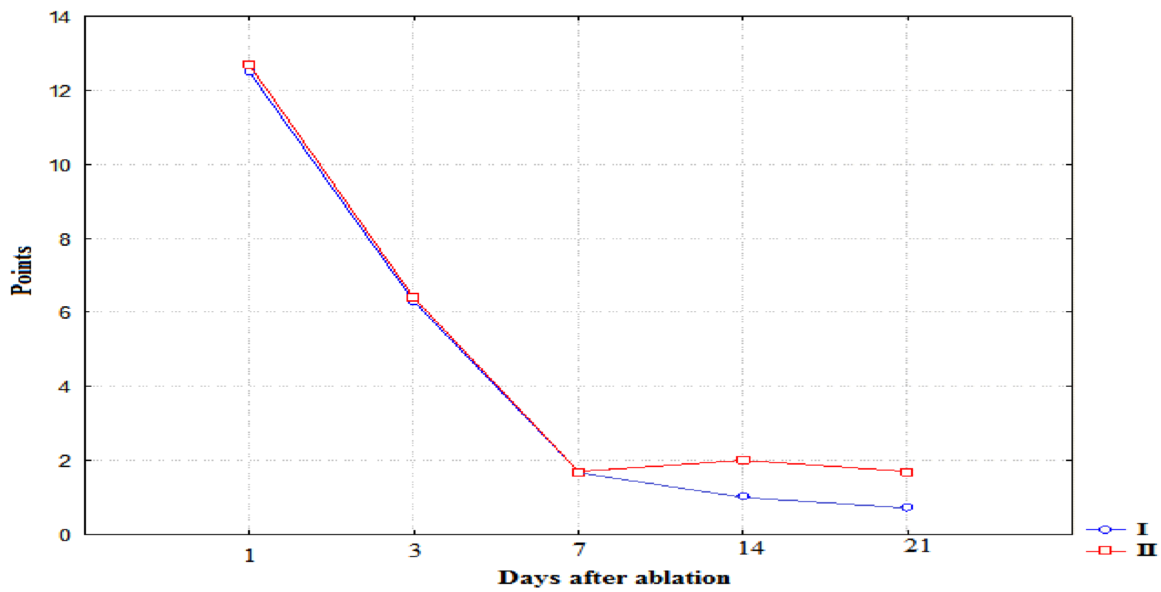

Fig. 2. The distribution curve of the average values (M) derived from the assessment of the rabbits eyes clinical condition (27 - point scale) after transepithelial photorefractive keratectomy (-3.5 sph correction, 50-micron central depth) with the preliminary riboflavin saturation of the stroma (I) and without such saturation (II).

The saturation of corneal stroma with Riboflavin did not affect the accuracy of the photorefractive ablation. At this point there is a lack of difference in the residual corneal thickness after ablation with Riboflavin and without it, according to the optical coherence tomography studies, in both series of experiments (table 2, figure 2).

DISCUSSION

Several factors indicate the effectiveness of the addition of Riboflavin for the photoprotection in keratorefractive laser surgery. Firstly, Riboflavin has antioxidant properties. It is involved in the suppression of an oxidative stress in all tissues of the body [8]. Oxidative stress with a cascade of photobiological effects, which occur in keratocytes of the cornea after excimer laser ablation, is similar to when the human skin cells are exposed to UV radiation. Secondly, saturated with Riboflavin stromal layers of the cornea function as spectral filters and block UV radiation. It protects keratocytes from the ablation-induced secondary UV radiation in the deeper layers of the corneal stroma. Thirdly, the absorption spectra of Riboflavin have multiple peaks with a maximum extinction coefficient in the range of long (C) and medium (B) UV radiation. This fact is important, because in the spectrum of a secondary radiation, induced by the excimer-laser ablation, take place in the medium (B) and near (A) range of UV radiation [3-7].

In the ophthalmic literature there are numerous studies on the saturation of corneal stroma with Riboflavin when performing cross-linking [9-11]. The analysis of these studies showed that the optimum concentrations are 0.1% and 0.25%. Moreover, in cases of the accelerated cross-linking it is recommend to use of 0.25% solution of Riboflavin. In this regard, for the aerosol saturation of the cornea we chose a 0.25% solution of Riboflavin in the isotonic water solution. The use of the high concentration is not advisable, because it does not increase the saturation of a corneal stroma with Riboflavin [17].

Aerosol spraying technique provided a unique opportunity for creating a thin layer over the entire front surface of the eye with a uniform distribution in the precorneal tear film. Our attention was drawn to a method of producing an aerosol of the drug with the use of an ultrasonic nebulizer with innovative membrane-mesh technology of a solution dispersion. Ultrasonic vibrations provide ultra-thin dispersion (not achievable in the other ways), increasing the interphase surface of the reacting elements. In the process of the ultrasonic emulsification and dispersion increase the surface of interaction. According to the specific researches, it can significantly increase the saturation of tissue with a medicine [12]. As for the cornea, increases the possibility of diffusing Riboflavin into its stroma.

Cornea that is saturated with Riboflavin is a barrier for the penetration of the ablation -induced secondary UV radiation into the deeper layers of the stroma. This is due to the ability of Riboflavin to absorb UV photons. A secondary radiation induced by excimer laser ablation, provokes an oxidative stress in the cornea and an apoptosis of keratocytes in the stroma layers, adjacent to the zone of ablation. All this affects for the development of the postoperative aseptic inflammatory reaction, regeneration and a final refractive effect. That's why there is no doubt in the appropriateness of using Riboflavin for freezing the ablation-induced secondary UV radiation, which is determined by the concept of photoprotection.

The choice as a technology model of transepithelial photorefractive keratectomy for the evaluation of a photoprotection of Riboflavin was not accidental. This operation is performed only contactless by a laser beam, and the biomechanical properties of the cornea are violated to a lesser extent than with a laser surgery of in-situ Keratomileusis [13-19]. That is why there's no doubt in the desirability of developing new approaches for the optimization of the technology for transepithelial photorefractive keratectomy [8]. One possible approach is the use of a photoprotection with Riboflavin for reducing the effects of the ablation-induced secondary UV radiation [13-15] on the keratocytes and under the zone of ablation - the layers of the corneal stroma [20,21]. Currently, according to the accuracy achieved by the effect of an excimer laser radiation with a wavelength of 193 nm is the leading approach among of all technologies of the refractive operations on the cornea. The data obtained about the photoprotection, after the saturation of stroma with Riboflavin in the transepithelial photorefractive keratectomy, indicate such technology of a refractive ablation of the cornea as promising. This technology of keratoablation may find its application in operations such as Epi LASIK, LASIK, photorefractive keratectomy and Femto LASIK.

CONCLUSIONS

- Performing a photorefractive ablation, after pre-saturation of the corneal stroma in 0.25% isotonic solution of Riboflavin, provides photoprotection from the secondary UV radiation induced by the ablation, which reduces the severity of the corneal syndrome, reduces the inflammatory response and accelerates the onset of a full epithelialization.
- Performing the excimer laser ablation of the corneal stroma, in 0.25% isotonic saturated solution of Riboflavin, does not influence on its residual thickness, which is an important indicator of the accuracy of the refraction profiling of the cornea.

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