Use of keratoxenoimplant for therapeutic and therapeutic-and-tectonic keratoplasty in severe ocular burns and corneal ulcerations of various etiologies

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Background: *The acute shortage of human donor corneas for transplantation has emerged, setting ophthalmologists a task of searching for new graft materials for use in keratoplasty.*
Purpose: *To investigate the possibility of using the keratoxenoimplant for therapeutic or therapeutic-and-tectonic keratoplasty in ocular burns or ulcerative sequelae of corneal inflammatory diseases.*
Materials and Methods: *A clinical study of the use of the keratoxenoimplant was performed in 69 eyes (60 patients) with ocular burns or ulcerative sequelae of corneal inflammatory diseases.*
Results: *In all eyes of the study, the keratoxenoimplant was found to facilitate the preservation of the globe and light perception. In some patients, following a number of rehabilitative-and-reconstructive procedures, we managed to implant the keratoprosthesis and to restore sight (in some cases to the visual acuity of 0.5).*
Conclusion: *The results provide the basis for recommendation of the keratoxenoimplant for wide use if human donor material is not easily available.*

Introduction

Corneal diseases and corneal damage are one of the leading causes of blindness worldwide, with keratoplasty being the main method of treatment. Even in a number of the developed countries, however, the need for human donor cornea far exceeds the supply [1]. In some Asian and African countries, where cultural and religious concerns hamper or even forbid receipt of corneas from human cadavers, the shortages of donor cornea are especially acute [1].

The introduction of recent organ and tissue transplantation legislation in Ukraine also has resulted in increased problems for national transplantations in general and ophthalmic transplantations in particular. The acute shortage of human donor corneas for transplantation has emerged, setting ophthalmologists a task of searching for new graft materials for use in keratoplasty.

In our opinion, animal corneal xenografts can serve as a source for this purpose. The experiments to investigate the possibility of using corneas from pigs, sheep, dogs, rabbits etc for transplantation into humans [2-6] were conducted as early as the 19th century, and, in the 20th century, they were expanded to include corneas from gibbons, cows and fish [7-9]. In spite of variability of corneal xenografts proposed, they failed to get acceptance in clinical practice. Voino-Iasenetskii [10] conducted large-scale experimental corneal "heteroplasty" (xenotransplantation) studies involving different animals at the Filatov Institute. Those studies revealed that vast majority of xenografts became opaque and vascularized (or lysed and rejected) due to severe histoincompatibility response. In a medical (including ophthalmic) community, there is currently renewed interest in using animal organs and tissues for transplantation into humans [1].

Porcine cornea has become a source for our graft material of choice for keratoplasty, since there are many similarities between pigs and humans in regard to anatomy and physiology [11-13]. Additionally, the porcine cornea has been found sufficiently similar to the human in structure and biomechanical parameters [14-18]. Immunologically, the pig is, however, less preferable than the primate as a source for corneal grafts due to the genetic differences between pigs and humans. Nevertheless, since the cornea is an immune-privileged tissue and is not immediately vascularized, its fate as a xenograft is supposed to be better than other organ xenografts [19-20].

Initially, we conducted multidisciplinary experimental studies to investigate the effect of different tissue preservation techniques (moist chamber technique by Filatov, lyophilization, cryoprotectant-free cryopreservation at -180 єC, and cryopreservation at -180 єC with cryoprotector and subsequent lyophilization) on the anatomical and morphological

structure of porcine cornea. Histology and electron microscopy studies have revealed that corneal structure is better maintained by cryopreservation with cryoprotector, and is scarcely affected by the subsequent lyophilization. Cryolyophilized keratoxenoimplants are fabricated based on the technology co-developed by the Horbachevsky Ternopil State Medical University and Filatov Institute of Eye Diseases and Tissue Therapy (Patent 52278 U, 2010) [21]. The following technological stages are involved in the fabrication, allowing for durable storage of the products and making them readily available for keratoplasty: removal of the cornea from freshly slaughtered pig eye, proper treatment with cryoprotector, cryopreservation at -196 °C, vacuum drying, quality inspection, product packing and radiation-based sterilization.

Additionally, the animal study was conducted to investigate (1) the reaction of recipient corneal tissue to intralamellar cryolyophilized porcine cornea implantation (with the implant cryopreserved at -180 єC with cryoprotector and subsequent lyophilization) and (2) the changes in the implant [21, 22]. The study involved both the intra- and interspecies transplantations (allo- and xenotransplantations, respectively), with porcine corneas transplanted to the corneas of Vietnamese pot-bellied pigs and rabbits, respectively. Clinical investigation of the reaction of the whole porcine eye and cornea to allotransplantation has shown that, likewise in intralamellar allotransplantation in humans, no immune response was observed. In intralamellar xenotransplantation, the reaction of the eye was insignificant and lasted 7-10 days, and later on the eye appeared normal; in some cases, the growth of isolated vessels from the peripheral cornea toward the implant was revealed. Both in allotransplantation and xenotransplantation, the clarity of the implant was maintained during a 6-month follow-up. In the former, histology did not reveal any significant changes in implant and corneal tissues, whereas in the latter, the formation of a delicate capsule around the implant was observed, the implant tended to resorb, and T cell-mediated immune response was insignificant. The promising results of experimental studies on the morphology and biophysiology of the keratoxenoimplant allowed us to proceed to its clinical use and studies.

The purpose of the study was to investigate the possibility of using the keratoxenoimplant for therapeutic and therapeutic-and-tectonic keratoplasty in ocular burns and ulcerative sequelae of corneal inflammatory diseases.

Materials and Methods

We retrospectively analysed the outcomes of 60 patients (69 eyes) who underwent therapeutic or therapeutic-and-tectonic keratoplasty with the cryolyophilized keratoxenoimplant (keratoxenoimplant medical product registered with the Ukrainian Ministry of Health, Reg. No. 9967 /2010) and were followed up at the Burn Department of the Filatov Institute during 2010 to 2014.

For the purpose of analysis, patients were divided into two groups. Group 1 comprised 48 eyes (39 patients, 34 men and 5 women) with ocular burns of different etiology (6 eyes and 42 eyes with thermal and chemical injuries, respectively) and severity (grades 3b and 4b by Iakymenko). Group 2 comprised 21 eyes (21 patients, 10 men and 11 women) with ulcerative sequelae of corneal inflammatory diseases (herpetic, tuberculous, bacterial, neurotrophic and neuroparalytic keratites, etc.).

In group 1, 3/48 eyes (6.2%) and 45/48 eyes (93.75%) presented with corneal perforations and extensive or total corneal ulcerations of various depths without perforations, respectively. Most of eyes with burn-induced damage had extensive conjunctival necrosis requiring for combined keratoplasty, conjunctival plasty and blepharorrhaphy. In 2/48 eyes (4.2%) and 33/48 eyes (68.7%), initial visual acuity (VA) was 0 and light perception (with accurate or inaccurate light projection), respectively. Additionally, in 13/48 eyes (27.1%), initial uncorrected visual acuity (UCVA) was 0.005 to 0.05. Preoperatively, elevated intraocular pressure (IOP), hypotony and IOP within normal limits were found in 9/48 eyes (18.75%), 13/48 eyes (27.8%) and 26/48 eyes (54.17%).

In group 2, 13/21 eyes (61.9%) and 8/21 eyes (38.1%) presented with corneal perforations and total corneal ulcerations of various depths without perforations, respectively. In 3/21 eyes (14.3%) and 11/21 eyes (52.4%), initial VA was 0 and light perception (with accurate or inaccurate light projection), respectively. Additionally, in 7/21 eyes (33.3%), initial UCVA 0.005 to 0.03, respectively. Preoperatively, elevated IOP, hypotony and IOP within normal limits were found in 2/21 eyes (9.5%), 16/211 eyes (76.2%) and 3/21 eyes (14.3%).

All patients underwent a general clinical examination. When taking the history from the burn patient, the physician tried to elicit information on the type of offending agent, nature and circumstances of the burn event, time to first aid treatment, and the inpatient care institution where the patient had received treatment. The circumstances that had led to the development of corneal ulcers of non-burn etiology were investigated. After completion of purposeful history taking, every patient underwent an ocular examination including the following: examination with bifocal illumination, biomicroscopy of the cornea, anterior chamber, iris and lens, electrophysiologic examination (examination of the electrical sensitivity of the optic nerve and critical frequency of phosphene disappearance), and, in case of leukoma, examination of X-ray light phenomenon (to determine whether the eye had shape vision). Whenever possible, IOP was measured with Maklakov tonometer (weight, 10.0 g). In patients with altered corneal structure, corneal thinning or perforation, IOP measurement with Maklakov tonometer was contraindicated, and IOP levels were measured with transpalpebral tonometer IGD-02.

Preoperative informed consent was obtained from all patients.

In both groups of the study, patients were followed up for 1.5 months to 4 years.

Operation methodologies

In severe burns, deep corneal infiltrates, erosions and ulcers usually develop 2-4 weeks after injury, do not respond to treatment, and can result in corneal melting. In these cases, we use superficial therapeutic keratoplasty by Puchkovskaya (1987) [23-24]. The superficial therapeutic keratoplasty with keratoxenoimplant differs from the customary lamellar keratoplasty in scraping off just the epithelium but not the altered anterior corneal lamellae. With this done, the keratoxenoimplant comprising the anterior corneal lamellae is transplanted onto the freshly denuded surface of the affected host cornea. Therefore, all potentially healthy corneal elements of the recipient eye are preserved, which is of crucial importance for restorative and regenerative processes. In this technique, the superficial lamellar keratoxenoimplant serves as a therapeutic dressing, under which the restoration of the host cornea occurs. The superficial therapeutic keratoplasty is a safe procedure, since the corneal tissue of the affected eye is not excised, and, should keratoxenoimplant rejection occur, the state of the host cornea will not change, and the surgeon may redo the procedure. In severe and even extremely severe burns, and in corneal ulcerations of non-burn etiology, the integrity of the eye can be almost always maintained with superficial therapeutic keratoplasty, followed later, if required, by optical keratoplasty. We applied superficial therapeutic keratoplasy with keratoxenoimplant in eyes with superficial corneal ulcerations.

In extremely severe ocular burns, sometimes rather early after injury, melting of the anterior corneal lamellae can occur, resulting in deep corneal ulceration and severe corneal thinning, and a prompt intervention is required to prevent imminent corneal perforation. At the first signs of corneal thinning, we perform therapeutic-and-tectonic keratoplasty to strengthen the cornea. The type of keratoplasty for corneal ulcerations and defects is selected based on the defect depth and area. If the depth of the ulceration was less than 1/3 the thickness of the normal cornea, we cover the cornea with a superficial lamellar keratoxenoimplant after removal of necrotic corneal lamellae. In eyes with significant (> 1/3) corneal thinning or a deep ulcer without perforation, we perform therapeutic-and-tectonic keratoplasty. In severe corneal thinning, we perform superficial therapeutic-and-tectonic keratoplasty with full-thickness keratoxenoimplant and scleral rim. In deep corneal ulcers or perforations, we perform "tectonic keratoplasty with two grafts" by Puchkovskaya (1971). The first graft and the second lamellar graft are cut from the posterior and anterior keratoxenoimplant, respectively. The former is used to plug the perforation, whereas the latter is used to cover the damaged cornea and lock the plug in place.

In all the methods described above, the conjunctival flap was used to cover the keratoxenoimplant, thus facilitating the engraftment. In extremely severe ocular burns with extensive or total necrosis of the bulbar and palpebral conjunctiva, and corneal thinning or perforation, to preserve the globe, we perform combined therapeutic-and-tectonic keratoplasty, conjunctival plasty and blepharorrhaphy. In this approach, we remove necrotized bulbar and palpebral conjunctiva until bleeding indicates the presence of viable tissue, scrape or cut off necrotic corneal lamellae, and perform tectonic keratoplasty with full-thickness keratoxenoimplant with scleral rim. The latter facilitates immobilization of the implant with episcleral suturing. The remnants of viable conjunctival, subconjunctival tissue and Tenon's capsule are mobilized and sutured over the cornea. Then the edges of the eyelids are deepithelialized or cleared of necrotic tissue and sutured together.

Additionally, all patients received complex pharmacological treatment taking into account the severity and stages of the burn process or the etiology of corneal ulcers unrelated to burns. In recent years, we have been successfully using instillation of broad-spectrum antibiotics, floximed, tobrimed, medetrom, neladex, and, NSAID, clodifen (all produced by World Medicine Ophthalmics, Kyiv, Ukraine) to prevent infectious complications associated with therapeutic-and-tectonic keratoplasty. Postoperatively, active systemic anti-inflammatory (selective and nonselective NSAIDs) and antibacterial therapy was given. The use of powerful anti-inflammatory, anti-edematous, antiscarring, tissue-regeneration facilitating, and, if needed, fibrinolytic therapy in ocular burns allowed us not only to prevent the development of ocular hypertension, but also to prevent the development of secondary post-burn glaucoma in most of patients.

Surgical success was defined as healing of the ulcer, prevention of corneal perforation, and preservation of light perception. However, if perforation was present at admission, surgical success was defined as preservation of the eye and light perception.

Results and Discussion

In group 1, most of the patients, 28 (71.8%) and 11 patients (28.2%) were operated early (17±11.74 days) and late (4.1±2.9 months) after burn, respectively. Most of the latter patients had been referred to the Institute too late. In group 2, only 6 patients (28.6%) were operated early (14.3±9.97 days), whereas most of the patients (7 patients (33.3%) and 8 patients (38.1%)) were operated late (4.6±2.6 months and 1 to 8 years, respectively) after the onset of disease.

Mild inflammation and complete adaptation of surgical wound edges was observed early postoperatively in all patients. Late postoperative course was uneventful.

In transient ocular hypertension after burn injury or keratouveitis, or if secondary post-burn glaucoma developed, we used carbonic anhydrase inhibitors (2% dorzolamide hydrochloride (Dorzamed, World Medicine), in particular) to reduce IOP; these agents decrease the intraocular fluid production in such eyes.

Complete ulcer healing and closure of corneal fistula with the development of corneal haze at the defect site, and mild vascularization was observed in all 69 eyes (60 patients) by the time of discharge from the in-patient department. In all eyes with burns, the operation resulted in (a) prevention of corneal perforation and preservation of the globe if the eye presented without perforation; (b) preservation of light perception, if the eye presented without perforation, and (c) the potential for sight

restoration to those who had sight before the operation. Because the keratoxenoimplant covered the cornea completely in almost all postoperative eyes, a reduction in VA was observed in eyes which had preoperative shape vision. In group 1, VA postoperatively decreased to light perception with accurate or inaccurate light projection, and changed insignificantly in 10/13 and 3/13 eyes with preoperative shape vision, respectively. In group 2, VA postoperatively decreased to light perception with accurate light projection and did not change in 6/7 and 1/7 eyes with preoperative shape vision, respectively.

In the late follow-up of patients or after rehabilitative-and-reconstructive procedures (if any), the keratoxenoimplant was found to develop opacities with intensive neovascularization and replacement of the implant tissue with vascularized scar tissue. In rare cases, we observed the conditions requiring repeat keratoplasty. Thus, partial lysis of keratoxenoimplant was observed in 1 eye (2.1%) of group 1 in 2 months, and in 2 eyes (9.5%) of group 2 in 1.5 and 10 months, requiring repeat keratoplasty with keratoxenoimplant. It seems likely that more frequent lysis in group 2 can be explained by conjunctival dehiscence observed over the keratoxenoimplant in the late follow-up.

In most of eyes with post-burn damage, we had to combine therapeutic-and-tectonic keratoplasty with conjunctival plasty and blepharorrhaphy due to extensive conjunctival necrosis. This resulted in extensive symblepharon or ankylosymblepharon formation. Such cases require a number of rehabilitative-and-reconstructive operations involving labial mucosa grafting to preserve sight or to make the conjunctival cavity suitable for fitting a cosmetic prosthesis. Rehabilitative-and-reconstructive procedures for symblepharon or ankylosymblepharon have been already performed in 13 eyes (27.1%) and 3 eyes (14.3%) of group 1 and group 2, respectively.

Although the globe and light perception in these eyes were preserved, previously, optical keratoplasty has shown little promise in such cases due to formation of a dense leukoma. Prosthetic approaches are currently the only hope for such patients to recover some sight. Thus, in 5 eyes (10.4%) with sequelae of extremely severe burns, a number of rehabilitative-and-reconstructive procedures were followed by keratoprosthesis implantation. This allowed us to recover some sight in all eyes, with a mean VA of 0.2 ± 0.2 (0.07-0.5). Usually, the low vision after keratoprosthesis surgery resulted from partial atrophy of the optic nerve due to the development of secondary post-burn glaucoma or presence of retrokeratoprosthetic membranes with various densities. If a patient had a fellow eye with high VA, rehabilitative-and-reconstructive procedures were performed to restore the conjunctival fornices, thus preparing a site for the cosmetic prosthesis.

Conclusion

Therefore, the clinical study showed that the keratoxenoimplant is rather effective for therapeutic and therapeutic-and-tectonic keratoplasty in ocular burns and ulcerative sequelae of corneal inflammatory diseases. In all eyes of the study, the keratoxenoimplant facilitated the following: (a) healing of the ulcer, (b) preservation of the globe, and (c) preservation of light perception, if the eye presented with it. Additionally, in some patients, keratoprosthesis implantation was performed following a number of rehabilitative-and-reconstructive procedures, and allowed restoring some vision.

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