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Combined Use of Amniotic Membrane and Pyo-Bacteriophage in Clinical Ophthalmology

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ABSTRACT

Antibiotic resistance is recognized as a global problem nowadays, and phage therapy is indispensable. We decided to use cryopreserved amniotic membrane impregnated with liquid bacteriophage during the surgical treatment of corneal and mucosal diseases in antibiotic-allergic and antibiotic-resistant patients. We examined the postoperative antimicrobial effect of a pure amniotic membrane compared to an impregnated amniotic membrane (stored in the Pyo-bacteriophage for 30 minutes) in 54 ophthalmologic patients with a history of antibiotic resistance or allergy. Laboratory studies revealed the superior antimicrobial effect of the impregnated amniotic membrane over non-impregnated amniotic membranes.

Keywords: Allergy to antibiotics; amniotic membrane; antibiotic resistance; impregnation; phage therapy; Pyo-bacteriophage.

INTRODUCTION

According to world statistics, blindness caused by corneal pathology is one of the most significant and relevant health issues.[1,2]

Scarring and vascular changes develop in the cornea, while the neural receptors of the eye are healthy, leading to lifelong disability. In such cases, keratoplasty was the only solution until the amnion appeared on the scene. The results of keratoplasty surgery are generally satisfactory, although corneal graft rejection reactions are common, which complicates the outcome of the surgical intervention.[3]

For several years, some corneal pathologies have replaced keratoplasty with amniotic membrane transplantation.[4-6] Indications for its use depend on the stage of the disease and the volume of the damaged area of the cornea. It is believed that after the transplantation of the amniotic membrane, a cessation of pathological vascularization, healing of scars, corneal opacity, recovery from inflammatory processes, and active regeneration of corneal tissue takes place.

The amniotic membrane (AM) or amnion is a thin membrane on the inner side of the fetal placenta. It consists of 5 layers: epithelium (of ectodermal origin), basement membrane, dense inner layer, mesenchymal fibroblastic layer, and spongy outer layer.[7-9] It is a secretory epithelium that produces biologically active substances that determine its properties: anti-inflammatory action, ability to stimulate regenerative processes, and inhibition of pathological neovascularization. It releases endothelins, which enhance the proliferation, migration, and differentiation of epithelial stem cells.[1,7,10,11]

In clinical ophthalmology, amniotic transplantation is performed to treat dystrophic and inflammatory conditions of the conjunctiva and cornea, neurotrophic ulcer, bullous keratopathy, corneal thermal and chemical burns, recurrent corneal ulcer, corneal perforation, myopic cone, Graft-versus-host disease (GvHD), pterygium, various corneal diseases with limbal stem cell deficiency (LSCD), and also to create a filtration bleb during the antiglaucoma surgical treatment.[12-17]

Amnion can impregnate; that is, it can absorb medicines (antibiotics, corticosteroids, antifungal drugs, etc.) and accumulate them in the area where there are adhesions, in our case, on the cornea and conjunctiva. Therefore, the amniotic membrane transplant, impregnated or saturated with these specific medications during instillations, has a more prolonged and efficient effect on the damaged area than if administered without the amnion.[5,11]

Currently, several methods of amnion preservation are recognized by the FDA:

- Biotissue storage under hypothermic conditions (Fresh, stored at +4^{0}C). The tissue stored under these conditions preserves its biochemical and histomorphological

structure as much as possible, but the maximum shelf life of "Fresh" amnion is only two weeks;[7,8]

- Lyophilization (Freeze drying) was carried out for 24 hours at -40^{0}C on a special device. However, amnion preserved under such conditions loses its biochemical properties, and biologically active substances are no longer produced;[7,8,18]
- Dr. Tseng's preserved amnion, frozen at -800C, is the most popular method of obtaining an amniotic graft. In this way, the biochemical activity does not decrease in the preserved tissue, and the period of use is extended by 18 months.[7,18]

In 2013, we founded the first Amniotic Membrane Transplant Bank in Georgia. We currently obtain and preserve amniotic grafts using Tseng's modified method.

Amniotic membrane is obtained in the maternity hospital after cesarean section, based on the donor's informed consent.

We currently obtain and preserve cryopreserved amniotic membranes and use them extensively in eye surgery for various corneal diseases.

Since 2013, we have performed 545 surgical operations, covering the cornea with amniotic membrane. In 95% of these operations, we got a positive result, and in 15%, where the patients were found to be allergic and resistant to antibiotics, the postoperative treatment was prolonged. In such cases, Pyo-bacteriophage helped us solve this problem.

The World Health Organization (WHO) recognizes antibiotic resistance as a global problem threatening humanity, and bacteriophages play a significant role in its solution.

Bacteriophages

Phages (Greek: φάγος – "absorption") are viruses that selectively destroy bacterial cells.

Bacteriophages are the most numerous and widespread group of viruses. They can withstand temperature changes, drying, and freezing. Culturing bacteriophages is simple and characterized by a short generation period and a multiplicity of progeny.[5,19]

All the above necessitated the bacteriophage becoming a suitable model for scientific research.[5,19] One field in which bacteriophages are used is antibacterial therapy (phagotherapy), an alternative to antibiotics. Bacteriophages are also used to treat streptococcal, staphylococcal, and dysenteric infections.

Bacteriophage preparations were successfully developed in Georgia at the Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia. Founded in 1923 under the leadership of Giorgi Eliava, the institute is a pioneer in bacteriophage studies.[5,19]

Since the amnion can be impregnated, we decided to use the amniotic membrane impregnated with liquid Pyo-bacteriophage during the surgical treatment of patients who are allergic and resistant to antibiotics.

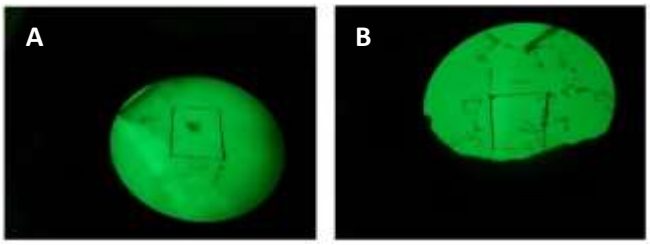
CASES

Amnion impregnation

To carry out amnion impregnation, the cryo-amniotic membrane was washed off preservatives with saline solution (NACL 0.9%), placed in Pyo-bacteriophage for 30 min., and washed again with saline solution.

During the histomorphological investigation, the cellular composition of the non-impregnated and impregnated amniotic membrane (AM) was compared using Transmission Electron Microscopy (TEM). Pyo-bacteriophage cells were visible in the tissue of the impregnated amniotic membrane (FIGURE1.B), while they were not present in the non-impregnated amnion membrane (Fig.1).

FIGURE 1. Non-impregnated (A) and impregnated (B) amniotic membrane



During laboratory investigation, the advantage of the Pyo-bacteriophage-impregnated amniotic membrane is evident, as the lysis of microbial strains is significantly higher there. The effect of a cryo-amniotic membrane non-impregnated with Pyo-bacteriophage and an impregnated one on the strains of streptococcus, staphylococcus, and gonococcus was compared.

Clinical research was conducted for ten years. We performed surgical treatment, covering the cornea with amniotic membrane, on 545 patients. Among them, 35 were allergic to antibiotics, and 19 had antibiotic resistance. In these 35 cases, a cryo-amniotic membrane impregnated with Pyo-bacteriophage was used during the surgical treatment.

Figures 2-5 represent the cases of the positive antimicrobial effect of combined use of amniotic membrane and Pyo-bacteriophage in clinical ophthalmology.

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FIGURE 2. The case of viral corneal ulcer and corneal perforation

A. Before the surgery

B. 1.5 months after surgery

C. Six months after surgery (viz 0.9)

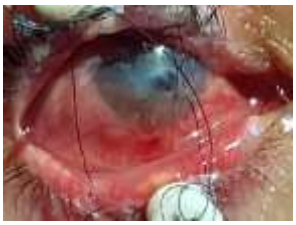
FIGURE 3. The case of viral corneal ulcer and corneal perforation with iris hernia

A. Before the surgery

B. Six months after surgery

FIGURE 4. The case of thermal burn of an eyelid, cornea, and limbus

A. Before the surgery

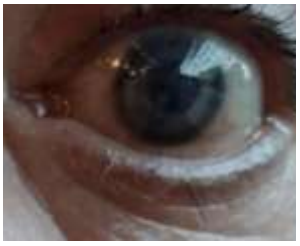


B. One month after the first surgery

C. Six months after the second surgery (vis 0.8)

FIGURE 5. The case of graft-host disease (GvHD)

A. Before the surgery



B. Two months after surgery (vis 0.7)

DISCUSSION

During the laboratory study, the effect of cryo amniotic membrane impregnated with Pyo-bacteriophage and non-impregnated one on streptococcus, staphylococcus, and gonococcus strains were compared. In the case of amnion impregnated with Pyo-bacteriophage, its advantage was evident - the lysis of microbial strains was significantly higher, proving that the amniotic membrane can be impregnated with liquid bacteriophage. During the histomorphological investigation, the cellular composition of the cryo-amniotic membrane impregnated with Pyo-bacteriophage and the non-impregnated one was compared using a biomicroscope. In the case of impregnated amnion, Pyo-bacteriophage cells were visible in the amniotic tissue, which confirms that the amniotic membrane can be impregnated with liquid bacteriophage. Over ten years, we have performed eye surgery (covering the cornea with a cryo-amniotic membrane) on 545 patients (DS: non-healing corneal ulcer). Of these, 35 patients had an antibiotic allergy, and 19 had antibiotic resistance. During their surgical treatment, a cryoamniotic membrane, previously kept in a pyro bacteriophage for 30 minutes, was used. Treatment and rehabilitation of the patients included 4-6 months, and in all the cases, we got positive results.

CONCLUSIONS

Studies have shown that cryo-amniotic membrane can be impregnated with liquid Pyo-bacteriophage and that liquid Pyo-bacteriophage-impregnated cryo-amniotic membrane is an effective alternative to antibiotic therapy. Its use during surgical treatment helps overcome the problems associated with treating antibiotic-allergic and antibiotic-resistant patients.

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