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Professional paper

The Expression of Interleukin-1 alpha, TNF and VEGF in Corneal Cells of Patients with Bullous Keratopathy

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A B S T R A C T

Bullous keratopathy (BK) is a chronic corneal edema with or without subepithelial bullae as a result of a loss of the endothelial cells. 15 patients with BK after cataract surgery with intraocular lens implantation, due to Fuchs dystrophy (n=3) or corneal endothelial trauma (n=12) were included in the study. All patients were treated by amniotic membrane transplantation (AMT). Corneal epithelial cells in patients suffering from BK secreted 3.91±3.09 pg/mL of IL-1 alpha, 4446±16.8 pg/mL of TNF and 81.43±37.81 pg/mL of VEGF-I. Levels of all 3 investigated cytokines were significantly higher as compared to controls (p<0.005). Amniotic membranes that were used to treat investigated patients contained 638.98±613.98 pg/mL of IL-1ra, 0.026±0.009 pg/mL of sTNF and 81.39±21.01 pg/mL of VEGF-R. Beneficial clinical effect of the AMT in treating BK could be explained by its natural production of pro-inflammatory cytokine antagonists such as IL-ra, sTNF antagonist and VEGF-R.

Key words: *amniotic membrane, bullous keratopathy, cytokines*

Introduction

Bullous keratopathy (BK) is a condition caracterized by chronic corneal stromal edema with or without subepithelial bullae, loss of transparency, due to lost of the endothelial cells. BK is often accompanied by rupture of bullae, persistent epithelial defects, scarring and neovascularization. Most frequently it is due to Fuchs' corneal endothelial dystrophy or endothelial trauma during cataract surgery with intaocular lens implantation. Fuchs' dystrophy is the most common corneal dystrophy, more often in women, causes billateral, progressive corneal endothelial cell loss[1–2]. Clinical findings vary from symptomatic Cornea guttata to decompensated cornea with stromal edema, subepithelial fibrosis and bulls in the epithelium. Give the characteristic appearance of the central »discharge« in the form of droplets in the endothelium, Descemet thin membrane and reduced number of endothelial cells. Clinical symptoms are pain, photophobia, epiphora, reduced visual acuity[3]. Corneal endothelial trauma can occur during intraocular cataract surgery or after placement of a poorly designed or malpositioned intraocular lens implant, leading to bullous keratopathy.

Recurrent erosion and persistent epithelial defects are results of continuously rupture of subepithelial bullae, exposing the cornea at times to infectious ulcers. In many corneal diseases there is the loss of immune privilege and the appearance of active suppression of intense immune reaction. There are findings from previous studies and in the studies in an animal model that proinflammatory cytokines interleukin IL-1a and tumor necrosis factor a (TNFa) play a role in the development of ocular inflammation[10–11]. Also corneal epithelial, endothelial cells, macrophages and active T cells produce VEGF, witch stimulates angiogenesis, connective tissue proliferation with scarring and corneal neovascularisation[12–13]. Many treatments are used to treat these conditions. Conventional treatmentis aimed to control inflammation and to protect corneal surface. It has been showen

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that amniotic membrane transplantation on the corneal surface improves healing of epithelium defects by preventing inflammatory cell infiltration and by reducing apoptosis in keratocytes.

Human amniotic membrane (AM) is the innermost layer of the placenta, consists of a layer of epithelial thick basement membrane and avascular stroma[3]. Healing effect of amniotic membrane has found application in many diseases of the cornea. Used as a graft or a patch the AM can promote healing of corneal disorders such as bullous keratopathy. Previous studies have shown that the beneficial effects of amniotic membrane transplantation may be due to the imunosuppressive efects of amniotic epithelial cells. The purpose whether amniotic epithelial cells secrete antiinflamatory factors.

We wanted to investigate the concentration of inflammatory cytokines corneal cells with BK and the concentration of anti-inflammatory cytokine secreted by the amniotic membrane and explain the effect of amniotic membrane to cure patients who do not respond to conventional therapy.

Participants and Methods

This prospective study included 15 patients with non-inflamatory corneal disease such as bullous keratopathy after cataract surgery with intraocular lens implantation, due to Fuchs' dystrophy (n=3) or corneal endothelial trauma (n=12) unresponsive to conventional treatment. We used corneal epithelial cells surrounding the defect. All patients were treated by amniotic membrane transplantation. Al the time of surgery, corneal cells were collected and analyzed for the presence of inflammatory cytokines (IL-1α, TNFα and VEGF) by ELISA (R&D System, USA). Production of pro-inflammatory cytokines antagonists (IL-1 ra, sTNF, VEGF-R) were detected from the remains of amniotic membrane used in transplants.

As a control group will used data from the literature from studies that have been doing the same research and as a control group used the cells of healthy donor corneas unsuitable for transplantation. Human tissue used in this study was obtained and managed in accordance with provisions of the Declaration of Helsinki.

AM was obtained shortly after elective cesarean delivery, with previously done serological tests that excluded HIV, human hepatitis type B and C and syphilis. The placenta was cleaned of blood clots with sterile Balanced Salt Solution c ontaining 50 mg/mL penicillin, 50 mg/mL streptomycin, 100 mg/mL neomycin and 2.5 mg/mL amphotericine B. The amnion was separated from the rest of the chorion by blunt dissection and flattened onto a nitrocellulose paper size of 0.45 mm, with the epithelium/basement membrane surface up, under lammelar--flow hood. AM were stored before transplantation at –80°C in sterile vial (Cornea Max) and glicerol at the ratio of 1:1. Prior to use in the operating room AM is dissolved, leaving the bottle at room temperature.

All patients receiving amniotic membrane had parabulbar anaesthesia with 2% lidocaine and corneal abrasion of epithelial cells surrounding the defect was done under the operating microscope. AM was placed and secured by interrupted 10–0 Vicryl sutures to the perilimbal conjunctiva. All patients received standard postoperative antibiotic treatment. AM dissolved over the period of two to three weeks after surgery.

The corneal cells that surround epithelial defect were debried and cultivated at 37°C for 24 hours, then stored at –80°C in the sterile bottles containing a nutrient medium (Cornea Max). The concentrations of IL-1a, TNFα and VEFG were measured in tissue supernatant using commercial available ELISA systems (ELISA-R&D systems, USA). The rest of the transplanted amniotic membrane was also cultured and stored at –80°C, and concentrations of IL-1ra, sTNRF and VEGF-R were measured in the supernatant of amniotic membrane. Corneal epithelial cells from a healthy donor from a eye bank were used as controls (n=10)[14].

For data processing we used the application program STATISTICA 7.1. Student t-test was used to compare the measurments. A p value of less than 0.005 was considered significant.

Results

Corneal epithelial cells in patients suffering from BK secreted 3.91±309 pg/mL of IL-1 alpha, 44.46±16.8 pg/mL of TNF 8143±3781 pg/mL of VEGF.

Levels of all 3 investigated cytokines were significantly higher as compared to controls (healthy epithelial corneal cells, 1.38±0.71 pg/mL of IL-1α, and the levels of TNFα and VEGF in healthy cornea cells were very small.) ($p<0.005$). The amniotic membrane that we used to treat investigated patients contained: 638.98±613.98 pg/mL of IL-1ra, 0.026±0009 pg/mL of sTNF 81.39±21.01 pg/mL of VEGF-R. Amniotic membrane transplantation facilitates rapid healing of corneal epithelium, reduces inflammation and pain, and stimulates epithelial cells re-growth.

Discussion

Corneal transplantation are nowdays resonable therapy for treating patients with bullous keratopathy. Better uderstanding of the pathogenesis os bullous keratopathy would allow us to predict and on timeeffectively prevent or cure affectide pathients. In previous studies has been experimentaly shown that amniotic membrane could be efficiently used for ocular surface reconstruction.

The amniotic basement membrane facilitates migration and growth of epithelial cells, therefore promoting epithelialization. The avascular stroma reduces fibrovascular ingrowth, and abnormal neovascularization. Am-

niotic epithelium produces anti inflamatory and growth factors such as interleukin IL-1 receptor antagonist, soluble TNF antagonist and VEGF-R. Its ability to modulate stromal scarring and its and its anti-inflammatory activity has lad to its use in the treatment of ocular surface pathology.

In this study we have found than the corneal epithelial concentration of cytokines IL-1α, TNFα and VEGF are significantly higher than in controls.

The amniotic membrane produce its natural antagonists such as IL-1ra, sTNF antagonist and anti VEGF-R.

Our resutls suggests that we could, with benefitial effects of amniotic membrane slow down or even stop formation of bullae, erosion,and new vesels in bullous kerathopathy.

Conclusion

Amniotic membrane transplantation is an efficient and safe treatment for bullous keratopathy. It has been shown to reduce the pain, promote corneal epithelialization and reduce conjunctival inflammation whereas in some cases it may also improve visual aquity.

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EKSPRESIJA INTERLEUKIN-1 ALFA, TNF ALFA I VEGF IZ STANICA ROŽNICE PACIJENATA OBOLJELIH OD BULOZNE KERATOPATIJE

SAŽETAK

Bulozna keratopatija (BK) je kronični kornealni edem sa ili bez subepitelnih bula, kao rezultat gubitka endotelnih stanica. U studiju je uključeno 15 pacijenata sa BK poslije operacije katarakte sa implantacijom intraokularne leće, od toga su 3 pacijenta bili sa Fuchovom distrofijom, a 12 pacijenata sa kornealnom endotelnom traumom.Kod svih pacijenata učinjena je transplantacija amnijske membrane (AMT). Stanice rožnice pacijenata sa BK izlučile su 3,91±3,09 pg/mL IL-1α, 44,46±16,8 pg/mL TNFα i 81,43±37,81 pg/mL VEGF. Razine svih triju ispitivanih citokina bile su značajno više u odnosu na kontrolnu skupinu ($p<0,005$). Amnijska membrana koju smo upotrijebili producirala je 638,98±613,98 pg/mL IL-1 ra, 0,026±0,009 pg/mL sTNF antagonista, te 81,39±21,01 pg/mL VEGF-R. Korisno djelovanje AMT u liječenju pacijenata sa BK može se objasniti lučenjem njenih prirodnih antagonista pro-inflamatornih citokina kao što su IL-1 ra, sTNF antagonist i VEGF-R.