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Professional paper

Transplantation of Amniotic Membrane in Corneal Ulcers and Persistant Epithelial Defects

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A B S T R A C T

Amniotic membrane transplantation (AMT) leads to reduction of inflammatory symptoms and causes faster epithelisation in corneal ulcers and persistant epithelial defect. 21 patients with corneal ulcer (n=18) or non-healing epithelial defect (n=3) unresponsive to conventional treatment were included in the study. All patients were treated by AMT. Corneal epithelial cells in patients suffering from corneal ulcer secreted 3.51±1.79 of IL-1a, 64.27±31.53 pg/mL of TNFα and 209.07±201.82 pg/mL of VEGF. Levels of all 3 investigated cytokines were significantly higher as compared to controls (p<0.005). Amniotic membranes that were used contained 775.69±613.98 pg/mL of IL-1ra, 0.036±0.033 pg/mL of sTNF and 175.01±166.63 pg/mL of VEGF-R. Supporting effect of the AMT could be explained by the fact that AM secretes its natural antinflammatory antagonists IL-1ra, sTNF and VEGF-R.

Key words: *amniotic membrane transplantation, corneal ulcer, persistent epithelial defects, cytokines*

Introduction

Microbial keratitis is serious ocular infection resulted in persistent epithelial defect or ulcer at the acute phase and corneal scarring and permanent vision loss at the chronic phase[1]. Healthy eye is covered with corneal and conjunctival epithelium. Corneal epithelium is known for its quick process of auto renewal, in the case of minor injury or inflammation. When the normal healing of corneal epithelium is disabled, pathological conditions are manifested as persistent epithelial defect (corneal ulcers and reccuring erosions), which can lead to significant vision loss[2]. Persistent epithelial defects are patology that, despite the fact that there are many possible treatments, often can not be solved without amniotic membrane transplantation (AMT)[3,4].

The most common indications for transplantation of amniotic membrane are injured ocular surface, drug-resistant ulcers, recurring erosion, bullous keratopathy and other corneal endothelial decompensation. Inflammatory reactions lead to the blurring of transparent media, corneal neovascularisation, tissue scarring and vision loss. In many inflammatory eye 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 cytokinesinterleukin IL-1α and tumor necrosis factor a (TNFα) play a role in the development of ocularinflammation[6–7]. Also corneal epithelial, endothelial cells, macrophages and active T cells produce VEGF which stimulates angiogenesis, connective tissue proliferation with scarring and corneal neovascularisation[9–11]. In animal models has also proven the possibility of treatment of certain inflammatory diseases of the eye anterior segment with inhibitors of inflammatory mediators[8].

Several mediators have been implicated to induce migration of inflammatory cells, for example IL-1α and TNFα. Previous studies showed experimentally on animal model that the use of preserved amniotic membrane graft is efficient in the corneal surface reconstruction after epithelial removal and it can promote healing of persistent corneal ulcers of different causes[7].

The aim of this study was to determine whether amniotic membrane with her natural antinflammatory activity can change course of the disease of persistent ep-

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ithelial defect. Also it will be investigated whether the inflammatory mediators interleukin 1α (IL-1α), tumor necrosis factor (TNFα) and endothelial growth factor (VEGF) stimulate inflammation and neovascularisation of anterior eye segment.

Patients and Methods

The prospective study included 21 patients with corneal ulcer (n=18) or persistent corneal epithelial defect (n=3) unresponsive to conventional treatment which lasted for more than 4 weeks. We used corneal epithelial cells surrounding the defect. We also used the remains of amniotic membrane used in transplants. As a control group used the cells of healthy corneas unsuitable for transplantation. The study didn't include patients with herpetic keratitis, and one that respond well to conventional therapy (antibiotic drops and ointments).

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 containing 50 mg/mL penicillin, 50 mg/mL streptomycin, 100 mg/mL neomycin and 2.5 mg/mL amphotericine B. The amnion wasseparated 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 debrid 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-1α, TNFα and VEFG were measured 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

Amniotic membrane transplantation was performed once in all 18 patiens. The tolerance was excellent in all cases. We observed no adverse effect. We observed complete healing in 19 weeks. Visual aquity improved in 15 patients and remained unchanged in 3 patients. Corneal epithelial cells in patients suffering from corneal epithelial defects secreted 3.51±1.79 pg/mL of IL-1a, 64.27± 31.53 pg/mL of TNFα and 209.07±201.82 pg/mL of VEGF. Levels of all 3 investigated cytokines were significantly higher as compared to controls (healthyepithelial corneal cells; IL-1α 1.38±0.71 pg/mL, TNFα and VEGF in very small levels) (p<0.005), (Figure 1). Amniotic membranes that were used to treat investigated patients contained 775.69±613.98 pg/mL of IL-1ra, 0.036±0.033 pg/mL of sTNF and 175.01±166.63 pg/mL of VEGF-R.

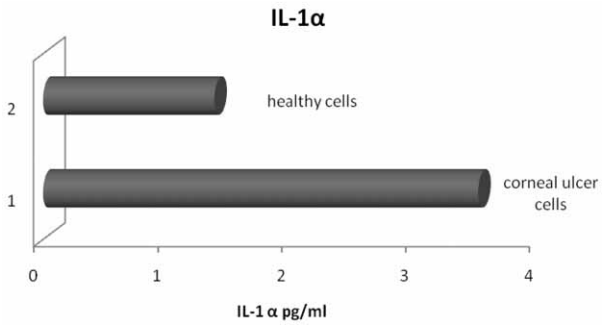

Fig. 1. Expression of interleukin-1α (IL-1α) in corneal epithelial cells in patients with corneal ulcer or persistent epithelial defects compared to controls.

Discussion

Amniotic epithelium produces anti-inflammatory and growth factors and has benefitial effect on treatment of inflamatory corneal diseases. It produces anti-inflammatory proteins such as interleukin (IL-1a) receptor antagonist, solubil TNFα, anti VEGF-R, hepatocite growth factor and trasforming factor â, facilitates the migration of epithelial cells, reinforcement of basal cellular adhesion and encouragement of epithelial differentiation. In previous studies it is shown that mouse corneas produced higher level of IL-1α and TNFα compared to the controls and some recent studies showed that this cytokines induce neovascularysation[14]. We have shown that human epitelial corneal cells produce proinflamatory cytokines IL-1a, TNFa and VEGF and AM produces its natural antagonists IL-1ra, sTNF and VEGF

It has been experimentally shown that AM could be efficiently used for ocular surface reconstruction because of its basement membrane and secretion of growth factors[5–14]. AMT leads to reduction of inflammatory symtoms by supporting adhesion and growth ofepithelial progenitor cells, causes faster epithelisation and decrease of neovascularisation. Stromal matrix of AM has

anti-scaring effect and it may promote nerve regeneration[14]. AM facilitates the migration and adhesion of epithelial cells, prevents epithelial apoptosis, causes rapid apoptosis of inflammatory cells[6–7]. We have shown that human epithelial corneal cells produce pro-inflammatory cytokines. Supporting effect of AMT could be explained by the fact that AM produces their natural antinflammatory antagonists.

Conclusion

The use of AM in patients with corneal ulcers and persistent epithelial defects, in whom conservative treatment had failed, seams a reasonable option, having in mind that IL-1α, TNFα and VEGF levels are increased in such eyes, and AM secrets their natural antagonists IL-1ra, sTNF and VEGF-R.

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TRANSPLANTACIJA AMNIJSKE MEMBRANE KOD ULKUSA ROŽNICE I PERZISTENTNIH EPITELNIH DEFEKATA

SAŽETAK

Transplantacija amnijske membrane (AMT) dovodi do brže epitelizacije i smanjuje upalnu reakciju kod ulkusa rožnice i perzistentnih epitelnih defekata rožnice. U našu studiju uključen je 21 pacijent sa ulkusom rožnice (n=18) ili sa necijeljećim epitelnim defektom (n=3), koji nisu reagirali na standardnu terapiju. Kod svih pacijenata izvršena je transplantacija amnijske membrane. Epitelne stanice rožnice pacijenata sa ulkusom rožnice izlučile su 3,51±1,79 IL-1a, 64,27±31,53 pg/mL TNFα i 209,07±201,82 pg/mL VEGF. Vrijednosti ispitivanih citokina u stanicama rožnice bile su statistički značajno veće u odnosu na kontrole ($p<0,005$). Iz supernatanta amnijske membrane izmjerena je koncentracija 775,69±613,98 pg/mL IL-1ra, 0,036±0,033 pg/mL sTNF i 175,01±166,63 pg/mL VEGF-R. Terapijsko djelovanje amnijske membrane se može objasniti time da ona luči prirodne protuupalne antagoniste IL-1ra, sTNF i VEGF-R.