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Tissue-engineered epithelium transplantation on corneal neovascularization in alkali - induced limbal stem cell deficiency

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Abstract

• AIM: To evaluate the clinical outcomes of tissue-engineered epithelium transplantation on decreasing corneal neovascularization in patients with limbal stem cell deficiencies caused by alkali burns.

• METHODS: A non-randomized, retrospective observational case series included 23 eyes of 19 cases with severe corneal alkali burns from 2006 to 2011 in our hospital. All of these eyes had complete limbal stem cell deficiencies. Ten cases (13 eyes) were performed with tissue-engineered epithelium transplantation, and 9 cases (10 eyes) were performed with amnion membrane transplantation. Vascularization was observed in all cases by slit-lamp microscope before and after surgery. A neovascularization scoring system was used to evaluate each eye at the 21th and 60th after surgery.

• RESULTS: At the 21th day and 60th day after tissue-engineered epithelium transplantation or amniotic membrane transplantation was performed, corneal neovascularization decreased significantly ($P<0.05$). And averaged scores of corneal neovascularization were significantly lower in tissue-engineered epithelium transplantation group at the 21th day and 60th day after surgery than those of amniotic membrane transplantation group($P<0.05$).

• CONCLUSION: Tissue-engineered epithelium transplantation is more effective in inhibitting the growth of corneal neovascularization in patients with limbal stem cell deficiencies caused by alkali burn than amnion membrane transplantation.

• KEYWORDS: corneal neovascularization; tissue-engineering; eye burn
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INTRODUCTION

Severe corneal alkali burn causing depletion of limbal stem cells leads to persistent epithelial defects and corneal opacification. Under the effect of inflammation chemokines, neovascularization grows from the edge of the cornea. Inflammation and the formation of neovascularization can affect the transparency of cornea and reduces vision. For severe corneal alkali burns, amniotic membrane can inhibit corneal inflammation. But it can not replace the function of the damaged or dysfunctional limbal stem cell. If the ocular surface could be covered with a large enough corneal epithelial sheet that included potential stem cells, then a smooth and clear ocular surface would be reconstructed more rapidly. In this study, employment of tissue-engineered epithelium transplantation is reported. The results for 23 eyes of 19 cases with severe alkali burns from 2006 to 2011 in our hospital are presented in this paper.

MATERIALS AND METHODS

Materials This study was approved by the institutional review board of the First Affiliated Hospital of General Hospital of Chinese PLA, in Beijing, China. We obtained written informed consent from all individuals. The study consisted of 23 eyes of 19 cases with complete limbal stem cell deficiencies (with complete disappearance of the palisades of Vogt) caused by severe alkali burns in our hospital from 2006 to 2011. The tissue-engineered epithelium transplantation group included 10 cases (13 eyes), consisting of 8 males (10 eyes) and 2 females (3 eyes) with a mean age of 43 years (range 25-58 years) and the past hours to hospital after burn were 6 to 48 hours. Tissue-engineered epithelium transplantation was performed at the 13th day (on average) after burn. Amniotic membrane transplantation group included 9 cases (10 eyes), consisting of 6 males (7 eyes) and 3 females (3 eyes) with a mean age of 39 years (range 30-49 years) and the past hours to hospital after burn were 2 to 50 hours. Amniotic membrane transplantation was performed at the 3th day (on average) after burn. All cases in two groups received nutrition support, conjunctival sac irrigation with saline, antibiotic eyedrops and/or eyegels (0.5% levofloxacin), artificial tears without preservatives four times a day.

Methods

Preparation of tissue-engineered epithelium Tissue-engineered epithelium was cultivated *in vitro* according to the previous studies[1,2]. A limbal biopsy specimen of approximately 1×1.5mm was obtained from the limbus of the healthy contralateral eye in unilateral corneal burn or a healthy area of the ipsilateral eye in bilateral corneal burn and was rinsed with phosphate group buffer solution for three times, digested with 0.5g/L pancreatin digested for 10 minutes, and neutralized

Table 1 Averaged corneal neovascularization scores $\bar{x}\pm s$

Groups	Before surgery	Day 21after surgery	Day 60 after surgery
TET group	3.69±0.480	1.46±0.776	1.85±0.801
AMT group	3.62±0.650	2.54±0.877	2.92±0.862

TET=tissue-engineered epithelium transplantation; AMT=amniotic membrane transplantation.

with 10% DMEM medium containing 10% serum medium. Then the tissues were centrifuged and co-cultured with cells in a Petri dish with 0.5mL cornea culture liquid, which consisted of Epilife culture solution(Cascade Biologics, USA) and cornea growth additive. The culture medium was refreshed every three days. The cultivated corneal epithelial cells were mixed with 300μL fibrinogen and blood coagulation factor centrifugation at 3500r/min for 30 minutes, at 4℃ to form tissue-engineered corneal epithelium for using within 12-14 days. All of the cells cultivated in the fibrin gel stained positively for keratin 3, a marker specific for corneal epithelial differentiation (as evidenced by the homogenous immunostaining of all the cells in the gel with the AE5 antibody, which recognizes keratin 3).

Preparation of human amnion membrane Amnion membrane was obtained, processed, and preserved as reported by Guo *et al*[3]. While being used, it was washed clearly with sterile saline, and put into 3MU/L gentamycin solution for 30 minutes.

Surgical procedure The conjunctival tissue from the cornea up to 3mm outside the limbus was removed and the subconjunctiva tissue was also removed after lidocaine injection was applied under conjunctiva. The cultivated autologous epithelium or amnion was secured onto the corneal surface with 10-0 nylon sutures.

Postoperation management After surgery, antibiotic eye drops and eyegels (0.5% levofloxacin), and artificial tears without preservatives were administered routinely four times a day for 3 weeks.

Observation of corneal vascularization Vascularization was observed in all patients by slit-lamp microscope at the 21th day and 60th day after transplantation. A neovascularization scoring system reported previously[4] was used to evaluate each eye by the same examiner before and after surgery. Scoring of the degree of corneal neovascularization depended on the maximum reach of the invasion of corneal neovascularization: grade 0, no neovascularization; grade 1, maximum reach less than one-third the distance between the limbus and corneal center; grade 2, maximum reach between one- and two-thirds the distance between the limbus and corneal center; grade 3, maximum reach more than two-thirds the distance between the limbus and corneal center; grade 4, maximum invasion reaching the corneal center.

Statistical Analysis Based on the CHISS package, averaged corneal neovascularization scores in these two groups were matched and evaluated with t-test. The level of significance was taken as $P<0.05$.

RESULTS

The corneal surfaces of all 13 eyes were free from epithelial defects 48 hours after tissue - engineered epithelium transplantation. Within the first 2-3 weeks, inflammation and vascularization regressed remarkably. And neovascularization kept stability 3 weeks after surgery. All donor eyes remained healthy. In amniotic membrane transplantation group, four of the 10 eyes showed persistent epithelial defects 2 weeks after surgery and corneal opacification increased. At day 21 and 60 after tissue-engineered epithelium transplantation or amniotic membrane transplantation was performed, corneal neovascularization decreased significantly ($P<0.05$). In most of the cases in tissue - engineered epithelium transplantation group, the cornea was clear with minimal neovascularization. In contrast, amniotic membrane transplantation group showed moderate neovascularization and opacity. The averaged corneal neovascularization scores are shown in Table 1. At the 21th day and 60th day after transplantation, scores for corneal neovascularization were significantly lower in tissue-engineered epithelium transplantation group than those of amniotic membrane transplantation group ($P<0.05$). Although the averaged corneal neovascularization scores at the 60th day after tissue-engineered epithelium transplantation were higher than at the 21th day, there was no statistical significance ($P>0.05$).

DISCUSSION

Severe cornea alkali burns leads to the infiltration of large amount of inflammatory cells and mediators, which can cause cornea neovascularization. Amnion can reduce the basic fibroblast growth factor (bFGF) which could promote corneal neovascularization (CNV). The mechanism of this depends on the high level of TIMP2 protein excreted or released by amnion. That can inhibit the migration and proliferation of vascular endothelial cell[5]. However, corneal renewal and repair are mediated by stem cells of the limbus. For limbal stem cell deficiency, amniotic membrane can not replace the function of the damaged or dysfunctional limbal stem cell and secure a corneal epithelial phenotype. In healthy corneas, the corneal epithelial stem cells are located in the limbal region[6]. As these stem cells migrate from the limbus into the center of the cornea, they differentiate into mature corneal epithelial cells. In a severely injured cornea, in which the limbal and central epithelia are both absent, the neighboring conjunctival epithelial cells invade the corneal surface and the eye becomes covered with an abnormal conjunctiva. This process is accompanied by persistent epithelial defects, stromal scarring, and neovascularization[7,8]. At present, limbal stem cell transplantation applied in clinical contexts mainly includes autologous or allogenic corneal limbal tissues and *in vitro* cultivated limbal stem cells[9-11]. A small limbal biopsy obtained from unaffected eye can be cultivated *in vitro* to become a large enough limbal graft[12,13]. Investigators have been evaluating bioengineered tissues for transplantation in an attempt to provide sufficient numbers of relatively pure corneal epithelial stem cells to recreate a more normal ocular surface[14-16]. Although the cultivated allocorneal epithelium

transplantation also provides a stable epithelial surface for grafts, there is a greater risk of rejection. Systemic immunsuppression is used to prevent allograft rejection[17]. Autologous cultivated limbal epithelium transplantation without immunosuppression can successfully regenerate the corneal epithelium and reduce vascularization, allowing to perform successful cornea transplantation and restore vision[18-20]. The cultivation of corneal epithelium might offer an alternative to patients with unilateral complete limbal stem cell deficiencies and a therapeutic chance to patients with severe bilateral corneal-limbal epithelial defects. In this study, autologous transplantation of corneal stem cells cultivated in a fibrin-based matrix can successfully recreate the corneal epithelium in patients with limbal stem cell deficiency. The corneal neovascularization decreased significantly in tissue-engineered epithelium transplantation group at the 21th day and 60th day after surgery as compared with those in amniotic membrane transplantation group. Tissue-engineered epithelium transplantation can inhibit the corneal neovascularization significantly and provide a basement for further penetrating keratoplasty of increasing visual acuity, which is a better method to treat complete limbal stem cell deficiencies caused by severe alkali burns than amniotic membrane transplantation.

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组织工程上皮移植对碱烧伤角膜新生血管的影响

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摘要

目的:评价组织工程上皮移植在碱烧伤角膜缘干细胞缺乏症中对角膜新生血管的抑制作用。

方法:回顾性非随机的病例研究。2006/2011 年我院收治的 19 例(23 眼)完全性角膜缘干细胞缺乏症的碱烧伤患者,10 例 13 眼行组织工程上皮移植,9 例 10 眼行羊膜移植。所有患者在手术前后均用裂隙灯观察角膜新生血管情况,在术后第 21,60d 对角膜新生血管进行评分比较。

结果:术后第 21d 和术后第 60d 组织工程上皮移植组和羊膜移植组角膜新生血管均较术前明显减少($P<0.05$),在术后两个评价时间点,组织工程上皮移植组平均角膜新生血管分数明显低于羊膜移植组。

结论:对碱烧伤所致角膜缘干细胞缺乏的患者,组织工程上皮移植抑制角膜新生血管的作用明显好于羊膜移植。

关键词:角膜新生血管;组织工程;眼烧伤