·Brief Report·

Effect of amnion membrane transplantation on corneal neovascularization in 10 patients with alkali burn

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Abstract

• By observing clinical cases, we studied the curative effect of amnion membrane transplantation on decreasing corneal neovascularization (CNV). It was a non-randomized retrospective case-control study. Among 17 cases (21 eyes) of third-degree alkali burns from 2007 to 2010, 10 cases (12 eyes) were performed with amnion membrane transplantation operation, and others were not. Amnion membrane transplantation was performed at the 3rd day after burn in the treatment group. Areas of CNV in double groups were measured at the 14th day and 60th day after burn. Area of CNV in the treatment group was (66.207± 7.251)mm^2 at the 14th day after burn, and was 18.27% lower than that in the control group. Area of CNV in the treatment group was (120.046± 13.812)mm^2 at the 60th day after burn, and was 11.35% lower than that in the control group. There was both statistical significance (P <0.05). Amnion membrane transplantation operation can inhibit the growth of corneal neovascularization induced by alkali burn.

• KEYWORDS: amnion; transplantation; eye burn; corneal neovascularization

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INTRODUCTION

Eyes always undergo the loss of corneal epithelium after alkali burn. Under the effect of inflammation chemokines and so on, neovascularization grows from the edge of the cornea, and then forms corneal neovascularization, which accordingly affects the transparency of cornea and reduces vision. Nevertheless, amnion can prevent eye epithelialization, inhibit the hyperplasia of fibrovascular tissue and the formation of neovascularization [1]. There have already been many studies about the effect of amnion membrane transplantation on diminishing corneal neovascularization, but most of what were limited on the animal experiments, lacking of clinical studies. The followings are the concrete conditions of 17 cases (21 eyes) of third-degree alkali burns from 2007 to 2010 in our hospital.

MATERIALS AND METHODS

Materials From 2007 to 2010 there were 17 cases (21 eyes) of third-degree alkali burns in our hospital, according to the criterion made by ocular trauma and occupational eye disease research team of China. The treatment group which was performed with amnion membrane transplantation included 10 cases (12 eyes), consisting of 7 males (9 eyes) and 3 females (3 eyes). The average age of the treatment group was 35.3 years, and the past hours before visiting hospital were from 1.5 to 24 hours. Another 7 cases (9 eyes) without amnion membrane transplantation belonged to the control group, consisting of 6 males (7 eyes) of and 1 female (2 eyes). The average age of the control group was 37.1 years and the past hours before visiting hospital were from 1.5 to 30 hours. Both of two groups routinely received symptomatic treatments including conjunctival sac irrigation with saline, levofloxacin and epidermal growth factor eye drops (four times a day), a holistic antibiotics and nutrition support. Amnion membrane transplantation was performed to the treatment group three days after burn. To the control group, the medical care was the same as to the treatment group except for amnion membrane transplantation.

Methods Fresh placenta was got from healthy caesarean section and what was infected with HIV, HBV, HCV, and syphilis was removed. Under sterile conditions amnion was separated from chorion, marked with stitch on epithelium, and washed with gentamycin solution (1MU/L). Amnion was immersed in the saline solution (penicillin 50mg/L, streptomycin 50mg/L, amphotericin B 2.5mg/L) for 15 minutes. Next, it was washed by pure saline repeatedly, put into sterile glycerin jar and kept at -80℃. While being used, it was cut into proper size, washed clearly with sterile saline, and put into 3MU/L gentamycin solution for 30 minutes. The

prepared amnion beyond one month should be discarded. The operation should be performed under a microscope. After regular sterilization and application of proparacaine hydrochloride eye drops, 20g/L lidocaine injection was applied under conjunctiva. Necrotic tissue was removed by blade, including cornea, conjunctiva around cornea, tissue under the conjunctiva and superficial sclera. Proper size of amnion was tiled on the cornea with epithelium up, and then, fixed on the superficial sclera through conjunctiva with 10-00 nylon suture. Suture position: At the direction of 12 o'clock, accounting to 12 stitches; 2mm away from edge of cornea. That should make the amnion membrane cling to the eye tightly. Finally, the operation eyes were coated with ofloxacin oculentum.

Eyes were observed by slit-lamp microscope at the 14th day and 60th day after burn. Length of corneal neovascularization and the accumulated hours of corneas circumference were measured, and then we could calculate the area based on Formula Robert: $S=C/12\times3.1416\times[r^2-(r-L)^2]$. C stands for the accumulated hours of cornea circumference of neovascularization, L stands for the length of neovascularization (which was measured by vernier calipers under the microscope. Those neovascularization which was longer, less curving, and perpendicular to the tangent line of edge of cornea should be picked up), r stands for radius of cornea.

Statistical Analysis Based on statistical analysis software SPSS 12.0, areas of neovascularization in these two groups were matched and evaluated with t-test. $P<0.05$ was considered statistically.

RESULTS

The area of neovascularization of the treatment group was 66.2±7.3mm^2 at the 14th day after burn, which was 18.3% lower than the control group whose was 81.006±10.387mm^2. There was a statistical significance ($P<0.05$). The area of neovascularization of the treatment group was 120.0 ± 13.8mm^2 in the 60th day after burn, which was 11.4% lower than the control group whose was 135.4 ±18.9mm^2. There was also statistically significant ($P<0.05$).

DISCUSSION

The main performance of cornea after third-degree alkali burns is lack of blood in limbus. Along with that comes the infiltration of large amount of inflammatory cells and mediators. If it's just cured conservatively, a lot of neovascularization will grow beyond the edge of the cornea. Amnion has the clinical effect on reducing inflammation, neovascularization and the form of scar. Since Davis (1990) firstly used amnion as biological dressing in place of skin to repair burn wound, amnion as a kind of convenient, easily used, cheep biomembrane with many other advantages has been widely popularized in clinical application, such as coating the burn wound, repairing defect, *etc*.

Many literatures have reported that amniotic medium has a significant effect on reducing the Basic Fibroblast Growth Factor (bFGF) which could promote corneal neovascularization (CNV). The mechanism of this at least partially depends on the high level of TIMP2 protein excreted or released by amnion. That can inhibit the migration and proliferation of vascular endothelial cell [3]. In addition, there are laminin, fibronectin, type Ⅳ collagen fiber and so on in amnion which can stimulate the differentiation and hyperplasia of epithelium, prolong the life of epithelial cells, maintain the form of clone and enhance the adhesiveness of epithelial cells [4]. Amnion is the ideal substance to support the growth of epithelium. It contains kinds of protease. It can help clear inflammatory mediators through controlling the corresponding protease [5,6]. At the same time, amnion can restrain the expression of transforming factor and signal transduction. Meanwhile it can inhibit normal fibroblasts differentiating into myofibroblast [7], consequently the overgrowth of corneal stroma and collagen induced by inflammatory cells and cytokines was avoided. Hence, it would promote wound healing without scar while ocular surface reconstructs. Alkaline matter can stay in the eyes stably for a long time due to the ability that it can respond with lipids and protein in tissues into the soluble protein component. Although being irrigated repeatedly in conjunctival sac or anterior chamber, eye tissue can still be harmed by alkaline. Nevertheless, fibroblast layer and spongy layer in amnion after rehydration contain a lot of mucus which can dilute and buffer alkaline. In this study, we finds that whenever at the 14th day or 60th day after burn, the area of CNV in the treatment group is smaller than that in the control group. The results imply that amnion membrane transplantation can inhibit the growth of CNV significantly, which is a kind of ideal method to treat early-stage serious alkali burns of eyes.

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