·Basic Research·

Effect of vitamin A on the conjunctival goblet cells of rat after corneal transplantation

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Abstract

• AIM: To investigate the effect of vitamin A on the conjunctival goblet cells of rat after corneal transplantation.

• METHODS: Rat graft rejection models of corneal transplantation were established. SD rats were receptor and Wistar rats were donors. After corneal allografts were performed, 48 SD rats were randomly divided into three groups, 16 rats in each group. Group A was blank control group; group B was treated by oculotect gel (containing vitamin A); group C was treated by 1g/L dexamethasone eyedrops. Besides, group D was normal unoperated eyes. Slit-lamp microscope was employed to record and compare rejection index (RI) of corneal transplantation. By HE, PAS staining of conjunctival histological sections and image analysis system, the number and morphology of conjunctival goblet cells were observed and analyzed between operation group and normal group.

• RESULTS: The HE, PAS staining detection showed that the number of conjunctival goblet cells in oculotect gel group, 1g/L dexamethasone eyedrops group and control group is lower than that in normal group after surgery ($P<0.01$). The number of conjunctival goblet cells in oculotect gel group and 1g/L dexamethasone eyedrops group is higher than that in control group ($P<0.05$). The number of conjunctival goblet cells in 1g/L dexamethasone eyedrops group is higher than that in oculotect gel group ($P<0.05$).

• CONCLUSION: The results indicate that vitamin A may inhibit the decrease of conjunctival goblet cells after corneal allograft rejection in rats.

• KEYWORDS: vitamin A; oculotect gel; corneal transplantation; goblet cells; rats

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INTRODUCTION

Tear film has an important role in maintaining the normal function of eye surface, and the abnormal tear film would cause disorders of eye surface. Early studies showed that tear film was composed of lipid layer, liquid layer and mucoprotein layer. Mucoprotein layer plays the most important role in three layers of tear film and mucoprotein is mainly secreted by conjunctival goblet cells, so whether the structure and function of goblet cells were normal or not would influence the function of tear film. The functions of eye surface would be affected, too. Studies about the factors that affect goblet cells and how to protect goblet cells of eye surface are important in decreasing diseases of eye surface.

Studies showed that vitamin A can play an important role in maintaining the health of ocular surface, and vitamin A deficiency would cause the loss of conjunctival goblet cells, conjunctival epithelium cornification, thickening and squamous metaplasia. It is not clear whether local application of vitamin A can protect goblet cells or not. For the sake of observing the protective function for conjunctival goblet cells by local application of vitamin A, we established goblet cells losing animal models by corneal transplantation to observe the protective function of vitamin A to goblet cells. Oculotect gel is a kind of artificial tears used for treatment of dry eye. Clinical observations showed it had good therapeutic effects on dry eyes [1,2]. The main components of oculotect gel are polyacrylic acid (carbopol 980) and vitamin A palmitate [3]. Therefore we investigate the protective function of vitamin A to goblet cells in ocular surface through the study of the protective effects of oculotect gel on goblet cells.

Table 1 Postoperative comparison of goblet cells in superior conjunctiva (mean±SD)

Group	Total number	Palpebral conjunctiva	Fornix conjunctiva	Bulbar conjunctiva
A	96.86± 5.12	23.43±3.31	57.57±5.09	15.57±2.23
B	105.14± 4.51	25.14±3.29	63.71±3.82	16.43±2.51
C	127.71± 7.30	30.14±3.93	79.43±3.78	20.43±3.78
D	139.71±15.53	31.57±3.05	83.14±3.76	25.71±3.40

Table 2 Postoperative comparison of goblet cells in inferior conjunctiva (mean±SD)

Group	Total number	Palpebral conjunctiva	Fornix conjunctiva	Bulbar conjunctiva
A	43.57± 5.74	12.14±0.90	28.23±3.03	3.29±2.14
B	54.57± 5.86	12.29±1.38	35.71±3.17	5.43±2.94
C	96.14± 5.18	20.29±3.82	53.50±4.97	24.29±3.64
D	109.57±11.48	22.71±3.90	59.43±4.08	25.14±3.63

Table 3 Postoperative comparison of rejection index in different groups (mean±SD)

Group	Clarity	Edema	Vascularization	RI
A	1.80±0.26	1.60±0.24	2.40±0.31	5.80±0.64
B	1.67±0.25	1.48±0.19	2.23±0.22	5.39±0.67
C	0.38±0.13	0.63±0.18	1.36±0.21	2.65±0.17

MATERIALS AND METHODS

Animals Rat models of allograft rejection were made by forty-eight SD rats weighed (180±10)g and twenty-four Wistar rats weighed (200±10)g. Then they were randomly divided into control group (group A) oculotect gel-treated (group B), 1g/L dexa- methasone eyedrops-treated (group C), and normal group (group D). Physiological saline was used in control group and normal group. The eyedrops was used 4 times a day in each group.

Procedure The routine penetrating keratoplasty was performed on the rats eyes. The corneal graft with a size of 3.25mm diameter was taken out from the Wistar rat. The corneal piece with a size of 3.0mm diameter in the middle area of the SD rat's cornea was taken out, too. Then the transplant was fixed by 10-0 nylon suture to the corneal bed of SD rat. Gentamicin 1×10^4U and dexamethasone 1mg were injected subconjunctivally after the surgery.

Pathological Preparation Enucleation was performed in all rats at 1 week postoperatively. Corneal surgical area with surrounding tissues were selectively cut as specimen and soaked in the 40g/L formaldehyde solution over 48 hours. Routine procedure were performed for HE and PAS staining. Conjunctival goblet cells were extracted by impression cytologic techniques for PAS staining.

Statistical Analysis Micro-photo analysis technique was applied to analyze the surgical area tissues pathologically in 5 spots from the surgical area randomly. The number of conjunctival goblet cells in unit area was calculated. Statistical analysis of the number of lymphocytes and mononuclear-macrophage in the experimental and control group was performed through SPSS 11.0 software.

RESULTS

Animals We established a successful animal model of corneal allograft rejection. The number of goblet cells in normal group was compared with that in control group, and results showed that the number of goblet cells in each part of conjunctiva in group A was obviously less than that in group D. The difference of the number of goblet cells between group A and D was statistically significant ($P<0.05$; Figure 1,4; Table 1,2).

Effects of Vitamin A on Corneal Allograft Rejection The corneal neovascularization (CNV) appeared at 2-5 days postoperatively in the experimental groups and control group. It appeared first in group A and B, later in group C. The corneal transplant edema could be observed in the early time after surgery, and then disappeared slowly. The rejection index (RI) of the corneal transplant was significantly lower in group C than group A and B ($P<0.05$). No statistical difference of RI was found between group A and B ($P>0.05$, Table 3).

Effects of Vitamin A on Goblet Cells The number of conjunctival goblet cells in group A and B was compared with that in group C and D. It showed that the number in group A and B was less than that in group C and D ($P<0.01$). Group comparison showed that the total number of goblet cells in superior and inferior area of conjunctiva was higher in group B than that in group A ($P<0.05$). Comparison of goblet cells number at different parts of conjunctiva showed that the number was less in group A and B than that in group C and D ($P<0.05$). The comparison of the number of goblet cells at superior and inferior bulbar and palpebral

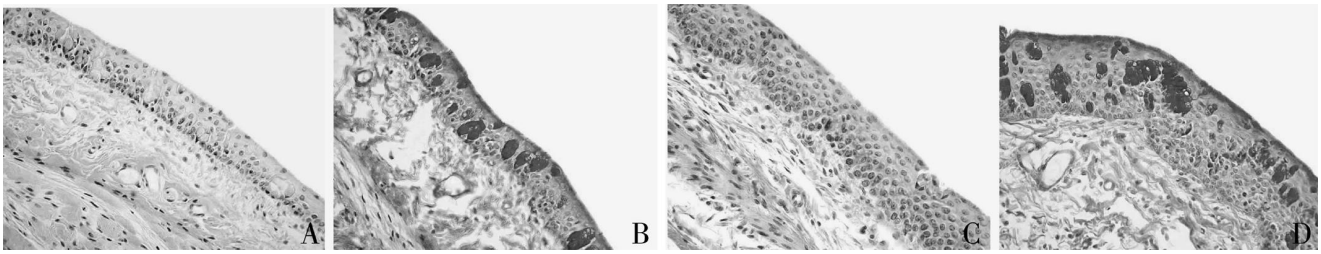

Figure 1 Goblet cells decreased obviously with lots of inflammatory cells infiltration in fornix conjunctiva in control group A,C: HE×400; B,D: PAS×400

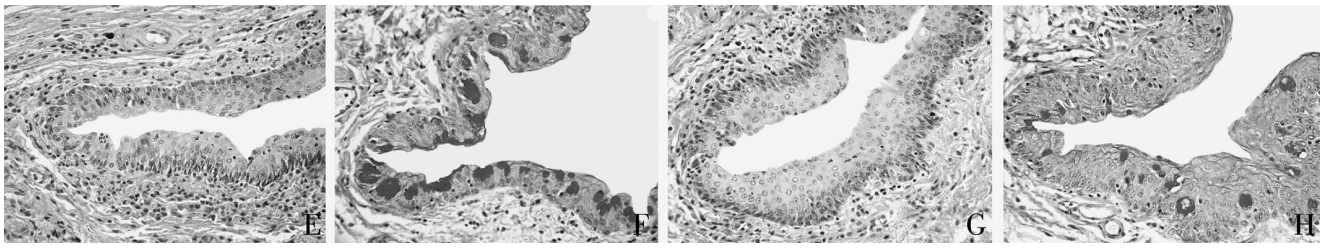

Figure 2 Goblet cells decreased with lots of inflammatory cells infiltration in fornix conjunctiva in oculotect gel-treated group E,G:HE×400; F,H:PAS×400

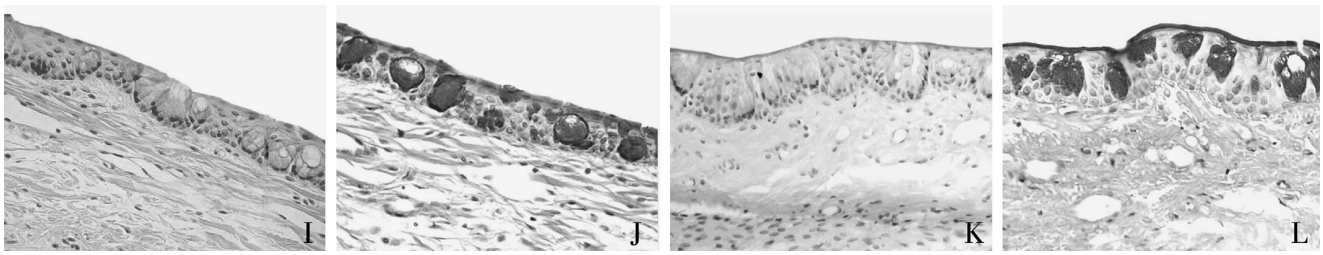

Figure 3 Goblet cells decreased slightly without obvious inflammatory cells infiltration in fornix conjunctiva in 1g/L dexamethasone-treated group I,K:HE×400; J,L:PAS×400

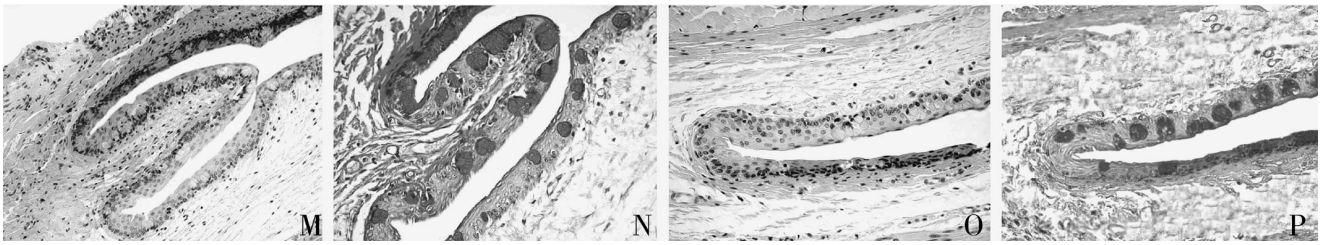

Figure 4 A large quantity of goblet cells exist in fornix conjunctival epithelium in normal group M,O:HE×400; N,P:PAS×400

conjunctiva showed no statistical difference between group A and B (P>0.05; Figure 1-4; Table 1,2).

DISCUSSION

Corneal allograft is a commonly used surgery manner in clinic. It can cure lots of serious corneal diseases, but the postoperative inflammatory reactions including corneal allograft immunological rejection would damage the normal surface of eyes[4,5]. Corneal allograft immunological rejection is complicated, while we made a successful corneal allograft immunological rejection animal model.

Conjunctival goblet cells are unicell mucilaginous glands in the conjunctival epithelium. Its main function is to secret MUC5AC (main component of slime layer of tear film). Maybe they can also secrete other mucoproteins such as MUC2 and MUC5B. Mucoprotein is the main component of innermost layer of tear film, which plays an important role in maintaining the normal function of eye surface. Any intrinsic and extrinsic factors that cause the decrease of the number and secretion of goblet cells would lead to dry eye and blurred vision [6]. We made an animal model of conjunctival goblet cells deficiency through corneal allograft immunological rejection. Results showed that the number of conjunctival goblet cells in each experimental group was obviously less than that in normal group, and the reduction of number in blank control group was most obvious. It indicated that the animal model of conjunctival goblet cells

deficiency was successful.

From the results we can also know that the extent of goblet cells decrease was different in different groups. The number of goblet cells was less in blank control group than that in any other groups. Next to the blank control group were oculotect gel-treated group and 1g/L dexamethasone eyedrops-treated group. Clinical observation by slit-lamp microscope showed that the acute stage of corneal allograft rejection was at 1 week after the operation. It indicated that maybe the remarkable decrease of goblet cells has a close relationship with the acute stage of corneal allograft immunological rejection. The decrease of goblet cells also occurred in oculotect gel-treated group, but the number of goblet cells was more than that in control group, which indicated that the oculotect gel had protective function to goblet cells.

Early studies showed that vitamin A participated in the synthesis of corneal glucoprotein and glycosaminoglycan in cornea and induced the cDNA synthesis increase of fibroblast in substantia propria layer. Vitamin A also participated in corneal energy metabolism and influenced conjunctival transdifferentiation, induced the increase of corneal endothelial epidermal growth factor receptor (EGFR) expression, which had enhancement effect on corneal wound healing induced by EGF. Vitamin A is essential in maintaining normal function of vision and immune integrity. Corneal epithelium couldn't grow normally and keratinocytes increased without sufficient vitamin A. Corneal basal layer also proliferated with cytodieresis and tenofibril and desmosome increased. The cells in the surface layer would be flattened and became anuclear keratinocytes which composed of kerato-hyaloplasm stuffed by kerato-fibril. Vitamin A deficiency will cause goblet cells loss in conjunctiva and conjunctival epithelium thickening, squamous metaplasia and cornification [7]. So the mechanism of protective function of oculotect gel to goblet cells is that goblet cells proliferated induced by expression of some factors and their receptors and inhibition of conjunctival epithelium cornification. The detail mechanisms still need further researches.

Oculotect gel was widely used in clinic for dry eyes. It was the 5th generation of artificial tears. Clinical investigation showed that oculotect gel was safe and effective in treatment of dry eye [1,2]. The main components of oculotect gel were poly- acrylic acid (carbopol 980) and vitamin A palmitate[3]. It has viscidity which can effectively increased the retention time in conjunctival sac by transition process of gel-water sample-gel when blinking. It can form a protective membrane lubricating the cornea, prolonging breakup time of tear film (BUT) and decreasing the mechanical friction of eyelids to corneal epithelium. Oculotect gel can inhibit the decrease of goblet cells caused by corneal transplantation not only because it contains vitamin A palmitate, but also because it plays an important role in forming a protective membrane on corneal surface and inhibiting the mechanical friction of eyelids to corneal epithelium.

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