·Basic Research·

Inhibitory effect of tetrandrine eye drops on corneal allograft rejection in rats

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Abstract

• AIM: To observe the effect of tetrandrine (Tet) eye drops of different concentrations on corneal graft and on allograft rejection in rats.

• METHODS: Models of allograft rejection were set up in 64 SD rats and they were then randomly divided into 3, 5, 10g/L tetrandrine eye drops-treated and control groups. At different times postoperatively, neovascularization and inflammation of corneal graft were observed using slit-lamp microscopy, HE staining, light microscopy and microphoto-analysis.

• RESULTS: The graft was infiltrated mainly with lymphocytes and mononuclear-macrophages. Corneal neovascularization and inflammation were significantly inhibited in the 5g/L Tet-treated group ($P<0.05$), compared with control group on day 7, 14, 21, 28 postoperatively.

• CONCLUSION: Corneal edema and corneal epithelial bubble appear when the graft is treated with tetrandrine of higher concentration (10g/L), but 5g/L Tet eye drops significantly inhibit corneal allograft rejection in rats without serious side-effects.

• KEYWORDS: tetrandrine; corneal allograft rejection; neovascularization; inflammation; rats

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INTRODUCTION

Corneal allograft immunologic rejection is the most serious complication of corneal transplantation in clinic and it can cause damage to the implant epithelium, inflammation of anterior chamber, iritis and corneal parenchyma edema. It may eventually lead to death of corneal allograft and failure of transplantation. The occurrence of corneal allograft immunologic rejection is about 23% and it is higher in the young patients than in the old. With a history of several corneal allograft transplantations or with intense neovascularization after transplantation, the occurrence of the rejection can be as high as 50% or more [1]. Against this background, ophthalmologists have always tried to find an effective, economical therapy with low side-effect. Tetrandrine (Tet) is a Chinese medicine. Early studies indicate that it has a wide range of pharmacological effects including diminishing inflammation, easing pain, lowering blood pressure and inhibiting fibroblast proliferation[2]. Thus we applied Tet eye drops of three concentrations to rats subject to experimental corneal allograft rejection and observe histopathologic and cytological alterations of the corneal graft.

MATERIALS AND METHODS

Animals Models of allograft rejection were established on SD rats (64), weighed (180±10)g, with corneal transplant from Wistar rats (32), weighed (200±10)g. Then the SD rats were randomly divided into control group (Group A) and 3, 5, 10g/L Tet eye drops-treated groups (Group B, C and D). The eye drops were applied 4 times a day in each group.

Procedure Routine penetrating keratoplasty was performed. Corneal transplant, 3.25mm in diameter, was obtained from the Wistar rat. Meanwhile, a piece, 3.0mm in diameter, was removed from the centre of the SD rat's cornea. Then the transplant was fixed by 10-0 nylon suture to the corneal bed of SD rats. Cidomycin 1×10^4U and dexamethasone 1mg were injected subconjunctivally after the surgery.

Pathological Preparation At 1st, 2nd, 3rd, 4th week postoperatively, 4 rats were enucleated respectively. Corneal surgical area with the surrounding tissue was selectively cut as speci-

Table 1 Corneal rejection index (RI) in each group ($\bar{x}\pm s$)

Group	Clarity	Edema	Vascularization	RI
Control	2.57 ± 0.53	2.29 ± 0.49	3.43 ± 0.79	8.29 ± 1.38
3g/L Tet[a]	1.93 ± 0.42	1.65 ± 0.61	2.87 ± 0.57	6.23 ± 0.98
5 g/L Tet[a]	0.63 ± 0.49	1.13 ± 0.53	2.12 ± 0.49	4.23 ± 0.53
10 g/L Tet[a]	1.69 ± 0.33	2.03 ± 0.84	1.85 ± 0.75	4.94 ± 0.70

[a]P<0.05 *vs* control group

men and soaked in formaldehyde solution (40g/L) over 48 hours. Routine procedure was performed for HE staining.

Statistical Analysis Micro-photo analysis was made to analyze the pathological alteration in 5 randomly-selected spots of the surgical area tissues. Inflammatory cells of different types in unit area were counted. Statistical analysis of the number of lymphocytes and mononuclear-macrophage in the experimental and control group were performed using SPSS 11.0 software.

RESULTS

Clinical Observation Corneal neovascularizaion (CNV) appeared on day 2-5 postoperatively in the experimental groups and control group. It appeared first in Group A and later in Group B-D. The corneal transplant edema was observed in the early stage after surgery and then disappeared slowly. The rejection index (RI)[3] of the corneal transplant was lower in experimental groups than in control group and it was the lowest in Group C at 2nd week after surgery (P<0.05, Table 1).

Cytological and Histopathological Alteration In Group A, at 2nd week after surgery, corneal transplant became thicker, infiltrated with lymphocytes and mononuclear-macrophage; at 4th week after surgery, large angiogenesis and scar formation was observed. In contrast, no obvious infiltration of inflammatory cells was observed in the experimental groups. The numbers of lymphocytes and mononuclear-macrophages in each experimental group and control group were compared at 1st, 2nd, 3rd and 4th week after surgery using t test involving two independent samples, and significant differences were shown, P<0.05, (Figures 1, 2). Compared with other experimental groups, Group D showed more remarkable corneal edema, with epithelial bubbles, in the whole process of the experiment.

DISCUSSION

Corneal allograft immunologic rejection is the most serious complication of corneal transplantation in clinic. Moreover its occurrence can be enhanced as a result of postoperative inflammation and corneal neovascularization [1]. So, it is of great value to find an effective, cheap and low side-effect therapy of corneal rejection.

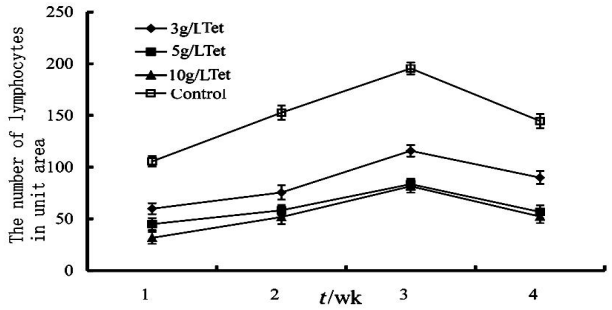

Figure 1 Comparison of the number of lymphocytes in each group

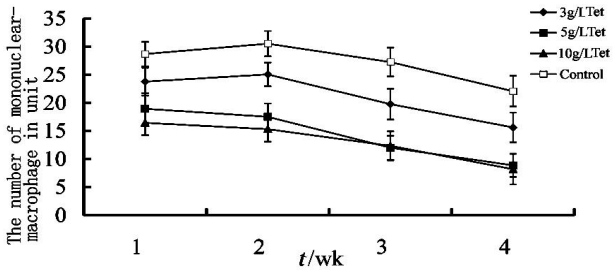

Figure 2 Comparison of the number of mononuclear macrophages in each group

Tet is a Chinese medicine. Early studies have indicated that it has a wide range of pharmacological effects including diminishing inflammation, easing pain, lowering blood pressure and inhibiting fibroblast proliferation [2], without obvious side-effects. Ophthalmic studies on animals have also proved that Tet plays some role in inhibiting immunologic inflammation, proliferative vitreoretinopathy as well as proliferation of retinoblastoma cells[4-6].

Thus in our experiment, we prepared Tet into eye drops, applied it at different concentrations to rats subject to experimental corneal allograft rejection and observed corneal histopathologic and cytological alterations over a relatively long period.

CNV appeared on day 2-5 after surgery in all groups. It appeared first in control group and later in experimental groups. Comparison of RI in different groups showed that it was lowest in 5g/L Tet group. Also, the corneal transplant

was infiltrated with a large number lymphocytes and mononuclear-macrophages. Compared with control group, proliferation of lymphocytes and mononuclear-macrophage was inhibited during the whole procedure in experimental groups, especially in 5g/L and 10g/L Tet group. It indicates that Tet has an obvious effect on inhibiting inflammation. Prolonged corneal edema and epithelial bubble was observed in 10g/L Tet group, which may indicate the potential toxic effect of Tet eye drops at high concentration on cornea. In contrast, such response was not observed in 5g/L Tet group. In the later stage of our observation, corneal rejection lessened and scarring took place in some cases. The possible explanation is that foreign antigen is blocked out, so there's no immunologic inflammatory reaction.

The findings above indicate that tetrandrine at concentration of 5g/L has an obvious inhibitory effect on CNV growth and immunologic rejection without serious side-effects. However, further research need to be made about the detailed pharmacological mechanisms of such effect and the effect of tetrandrine over prolonged application.

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