·Basic Research·

# Effect of Qingguangan on the expressions of MMP-2 and MMP-9 in filtering bleb after trabeculectomy in rabbits

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## Abstract

• AIM: To explore the effect of Qingguangan on the expressions of MMP-2 and MMP-9 in filtering bleb scarring area after trabeculectomy in rabbit model.

• METHODS: Thirty-two New Zealand rabbits were randomized into four groups: control group, experimental group, MMC group (ocular trabeculectomy in combination with MMC), and Qingguangan group. Trabeculectomy was performed on both eyes in each group except control group. Qingguangan group was mouth-fed with Qingguangan (solution). On postoperative day 14, the appearances of MMP-2 and MMP-9 on filtrating blebs were observed by immunohistochemistry.

• RESULTS: Statistical differences of the expressions of MMP-2 and MMP-9 were noted among groups on day 14 following surgery. Histology immunohistochemistry showed significant differences on the expressions of MMP-2 and MMP-9 between each groups($P$<0.05).

• CONCLUSION: Qingguangan can promote the expressions of MMP-2 and MMP-9.

• KEYWORDS: Qingguangan; trabeculectomy; MMP-2; MMP-9
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## INTRODUCTION

Filtration surgery is one of the most important operations for reducing intraocular pressure in glaucoma patients, and trabeculectomy is the best representation of the filtration surgery nowadays [1]. In recent two years, however, the failure rate of trabeculectomy is 15%-30%. The main reason for that is the excessive conjunctival fibroblasts proliferation in the filtration bleb [2]. Studies show that the leading cause for filtering bleb scarring is due to the excessive growth of Tenon's capsule fibroblasts (TCFS) and the accumulation of type Ⅰ collagen [3]. The Matrix metalloproteinase (MMP) family is involved in the breakdown of extracellular matrix (ECM) except polysaccharide [4]. Both MMP-2 and MMP-9 are members of MMP family, and they play vital role in the degradation of basilar membrane, wound healing and tissue repairing. The substrate of MMP-2 and MMP-9 are fibronectin elastin and type Ⅰ, Ⅲ, Ⅳ, Ⅴ, Ⅶ, Ⅹ collagen. Qingguangan is a natural Traditional Chinese Medicine composed by Huangqi, Shengdi, Dilong, Chishao, Honghua, Fulin, Cheqianzi and Baizhu (Chinese herbal medicine) which has been used on post-operation of trabeculectomy for 16 years. The aim of present study is to explore the effect of Qingguangan on increasing the expressions of MMP-2 and MMP-9 in filtering bleb.

## MATERIALS AND METHODS

**Materials** Mouse anti-human MMP-2 and MMP-9 monoclonal antibody, the practical concentration was 1:50. Immunohistochemical sp kit, DAB developer, neutral gum and other reagents were all provided by Wuhan Boster bio-engineering limited company. Qingguangan was provided by the Pharmacy Department of the First Affiliated Hospital, Hunan University of Traditional Chinese Medicine [Xiangweiyaoji (98)05No.029]. Thirty-two (different from the Chinese version of 32) healthy New Zealand rabbits, no restrictions on male or female, weighing 1.5kg-2kg, were provided by Hunan TCM University Animal Experimental Center. All animals were proved healthy after an examination using slit-lamp microscope and ophthalmoscope. The rabbits were randomly divided into four groups: blank (control) group, experimental group, MMC group (ocular trabeculectomy in combination with MMC), and Qingguangan group. All eyes were treated with Tobramycin eyedrops, three times a day, and three days before the operation.

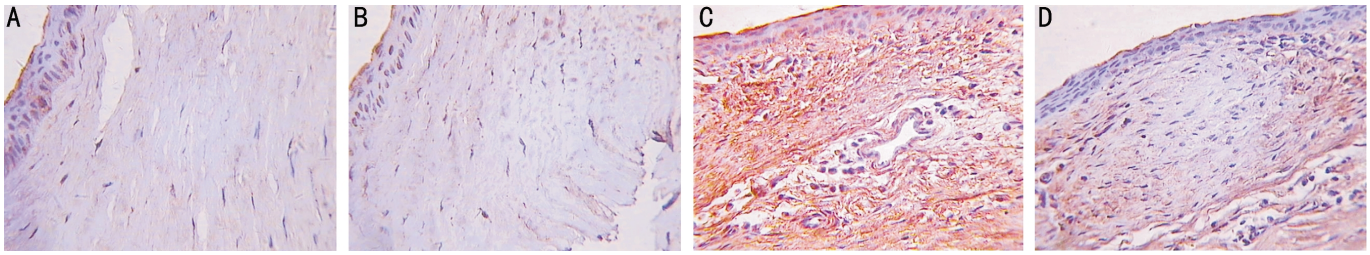


**Figure 1** A: MMP-2 negative expression in Tenon's capsule fibroblasts; B: MMP-9 negative expression in Tenon's capsule fibroblasts; C: MMP-2 positive expression in Tenon's capsule fibroblasts; D: MMP-9 positive expression in Tenon's capsule fibroblasts.

**Methods** The trabeculectomy operation was performed for both eyes. Rabbits were secured on the operation table after intravenously injected in their ear marginal mixture of distilled water, 3.2mL/kg and 0.8mL/kg Urethane. Along the limbus of cornea, a conjunctiva bleb based towards the conjunctival fornix was made on the temporal side of the superior rectus muscle. After separating the subconjunctiva tissue, exposing the surface of sclera, and stanching bleeding completely, a rectangular shaped sclera flap (3mm ×3mm), with 1/2 the thickness of the sclera was created in the 12 o'clock position. Its base pointed to the limbus of cornea. Then, the flap was separated till the 0.5mm inner transparent cornea limbus. In MMC group, a 4mm×4mm 0.5mg/mL MMC cotton disk was placed above the scleral flap. Pierce into the anterior chamber. Then the incision was enlarged, the deep sclera tissue (the location of trabecula) was cut and removed, size about 1mm×2mm, and periphery iris was resected. Finally, 1 stitch was applied at each disassociate angle of the bleb with 10-0 nylon thread, rearrange the conjunctiva bleb, 5-0 nylon thread to suture the conjunctiva bleb. Dexamethasone was injected into bulbar conjunctiva, and Tobramycin eyedrops was used three times daily for a week. Rabbits in Qingguangan group were fed with Qingguangan solution seperately for 2 weeks while the others were fed with distilled water 2 days after the operation.

**Histology analysis** On the 1st, 2nd and 4th weeks, two rabbits in each group were randomly chosen to be sacrificed by injecting air. Eyeballs were removed. Filtration blebs of 10mm×10mm were collected and treated with 4% parafo-rmaldehyde for 24 hours. The paraffin wax specimens slice continuously 4μm thick, and sections were dewaxed in xylene for 5-10 minutes and rehydrated through graded alcohol. Endogenous peroxidase activity was blocked by placing sections in 3% hydrogen deionized water for 10 minutes. In order to detect MMP-2 and MMP-9 epitopes, sections were placed in 0.01moL/L citrate buffer (pH 6.0) at 92℃ -98℃ for 20 minutes. To block nonspecific staining, slides were incubated in normal goat serum for 10 minutes. After three 5-minute intervals, slides were washed in PBS and were incubated overnight at 4℃ with primary antibodies, which were diluted at a ratio of 1:50. After three 5-minute intervals, sections were washed in PBS and were incubated sequentially with biotinylated rabbit anti-mouse IgG, followed by streptavidin combined *in vitro* with biotinylated horseradish peroxidase. After three 5-minute intervals, sections were washed in PBS, and the product was developed with diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin, dehydrated through graded alcohol, and mounted in resinous mountant. Sections were observed under light microscopy. They were being examined for the average optical density value of MMP-2 and MMP-9 in the TCFS by use of the HMIAS-2000 completely automatic medicine color image analysis system at 400 times of light microscope. Each section selects 5 fields of vision.

**Table 1 Expressions of MMP-2 and MMP-9 in Tenon's capsule fibroblasts**

| Groups | Cases | MMP-2 | MMP-9 |
|---|---|---|---|
| Control group | 5 | 0.338±0.036 | 0.310±0.066 |
| Experimental group | 5 | 0.334±0.056 | 0.392±0.054 |
| MMC group | 5 | 0.390±0.037 | 0.462±0.038 |
| Qingguangan group | 5 | 0.448±0.025 | 0.478±0.033 |

**Statistical Analysis** Measurement data among groups were compared using SPSS 16.0 software for analysis of variance. Results were expressed as mean±SD and analyzed by Normality test and ANOVA. $P$ <0.05 was considered as statistically significant.

**RESULTS**

**Expression of MMP −2 in Each Group of Tenon´s Capsule Fibroblasts** Positive representing MMP-2 and MMP-9 were appeared and stained with brownish yellow color (Figure 1C and 1D). Positive expression rates of MMP-2 were significantly higher in MMC group than those in experimental group ($P$ <0.05), while the difference between Qingguangan group and experimental group had reached a remarkably significant level ($P$ <0.01). The expression difference of MMP-2 between MMC group and Qingguangan group was not significant ($P$>0.05, Table 1).

**Expression of MMP −9 in Each Group of Tenon´s Capsule Fibroblasts** The expression difference of MMP-9 between Qingguangan group and experimental group was significant ($P$ <0.05), and the difference also existed

between MMC group and experimental group ($P$<0.05). However, the difference between Qingguangan group and MMC group was not significant ($P$>0.05, Table 1).

## DISCUSSION

Filtration surgery is the most commonly practiced operation for glaucoma[5]. The long-term success rate of this approach is only 75%, which is mainly attributed to the scarring of filtering bleb formed from the following stages [6]. In the early wound-healing stage, direct damage by surgery and local inflammation increases the chemotaxis of inocyte, such as TCFS to the surgery section, producing cytokine TGF-β1, TGF-β2 *etc* [7]. In the proliferation stage, TCFS has been activated and proliferated while TGF-β binds with cell surface receptors. In the rebuilding stage, TGF-β induces the proliferation of collagen by mechanocyte during scar formation.

Qingguangan is a natural Traditional Chinese Medicine composed by Huangqi, Shengdi, Dilong, Chishao, Honghua, Fulin, Cheqianzi and Baizhu (Chinese herbal medicine) which has been used on post-operation of trabeculectomy for 16 years. It has been proven to be safe and efficient in clinical applications [8,9]. Preliminary studies and literature research support that Qingguangan could also decrease IOP and reduce scarring effects in postoperative trabeculectomy patients by inhibiting apoptosis on pressurized human trabecular cell[10]. It has been proved that Qingguangan could reduce cell apoptosis of retinal structure and accelerate the removal of NO and glutaminic to protect optic nerve axons and retinal ganglion cells[11,12]. However,the exact underlying mechanism was unclear. In recent years, the matrix metalloproteinase has been shown to be the only enzyme that could breakdown the fibrous collagen heretofore [13]. At least 14 MMPs have been identified. Among them, gelatinase A (MMP-2) and gelatinase B (MMP-9), which belong to the gelatinase family, mainly degrade gelatin and IV, V, VII, X-type basal membrane collagen. They play vital role in the attachment of cells and the matrix [14]. It is shown that the decreased expressions of MMP-2 and MMP-9 may have caused not only collagen proliferation under conjunctiva, but also scarring in conjunctival sacs of open angle glaucoma patients[15].

In present experiment, Qingguangan was investigated for its effect on expressions of MMP-2 and MMP-9 and may possibly affect scarring. The present study showed that the positive expression rates of MMP-2 and MMP-9 were much higher in Qingguangan group and MMC group, but the expression difference of MMP-9 between MMC group and Qingguangan group was not significant. The results above indicated that both Qingguangan and MMC could promote the expressions of MMP-2 and MMP-9. But the mechanism of Qingguangan is still not clear and needs further investigation.

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