Filtering bleb area and intraocular pressure following subconjunctival injection of CTGF antibody after glaucoma filtration surgery in rabbits

Jian-Ming Wang, Na Hui, Ya-Zhi Fan, Lei Xiong, Nai-Xue Sun

INTRODUCTION

Glaucoma filtration surgery (GFS) is a very important operation for reducing intraocular pressure. After GFS, a filtering bleb will be established for aqueous humor drainage to subconjunctive tissue. The main reason causes the failure of filtering operation is the excessive conjunctival fibroblasts proliferation nearby filtering bleb, which lead to scarring. Studies show that connective tissue growth factor (CTGF), as the downstream factor of transforming growth factor beta (TGF-β) and the leading cause of filtering bleb scarring, can accelerate fibroblasts proliferation, migration, adhesion and secretion of extracellular matrix. However, the effects of CTGF antibody on filtering bleb area and intraocular pressure after GFS has not been reported yet. In this study, we establish rabbit glaucoma filtration operation model after trabeculectomy, and observe the postoperative filtering bleb area and intraocular pressure after subconjunctival injection of CTGF antibodies. Our aim is to discuss the role of CTGF antibodies on preventing scarring formation and provide the evidence for its clinical application.

MATERIALS AND METHODS

Materials

CTGF affinity purification antibody (200mg/L) from Wuhan Boster Bio-engineering Limited Company (Wuhan, China) was previously diluted to 50mg/L in sterile Eppendorf tube. IOP was measured with Kowa applanation tonometer (Model HA-2)(Tokyo, Japan). Five healthy New Zealand rabbits, no limit on male or female, weighing 1.5 to 2kg, are provided by Xi’an Jiaotong University Medicine Animal Experimental Center. All animal eyes were proved normal after examination with slit-lamp microscope and ophthalmoscope. All eyes were used by Chloramphenicol eyedrops 0.25%, four times daily, three days before operation. The IOPs of both eyes 3 times for each animal were tested with applanation tonometer, and the average value was used as the preoperation IOP.

Methods

Then the trabeculectomy operation were processed for both eyes, and all operations were accomplished by one operator. After Ketamine hydrochloride 50mg/kg and phenergan 10mg/kg mixed as anesthetic to be injected.
intramuscularly, rabbits were fixed on the operation table. Along the limbus of cornea, a conjunctiva bleb based towards the conjunctival fornix was made on the temporal side of superior rectus muscle. After separating the subconjunctiva tissue, exposing the surface of sclera, and stanching bleeding completely, a rectangular shaped sclera bleb (size 3mm×3mm), with 1/2 thickness of the sclera was created in 10:00-11:00 o'clock, its base point to the limbus of cornea, afterwards, separate the bleb till the 0.5mm inner transparent cornea limbus, 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 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.

One eye of each rabbit was randomly chosen as the control group immediately after the operation with 0.1ml PBS subconjunctival injection, and the other eye as the antibody group with 0.1ml 50mg/L CTGF antibody subconjunctively injection to the nasal side of superior rectus muscle. Apply eye-drops to the operation eye with Chloramphenicol ophthalmic solution postoperatively, 4 times per day, totally 1 week. 5 days after the operation, repeated the injection of PBS 0.1mL subconjuntivally to the control group whereas the antibody group with 50mg/L CTGF antibody 0.1mL. Observed the appearance of the filtering bleb with slit-lamp microscope, and measured the length and width of the bleb with vernier caliper, 3 times for each, and averaging to calculate the area of the bleb. Applanation tonometer was used to test the IOP of both eyes postoperative in day 1,3,5,7,10 and 14, each 3 times measurement for the averaged IOP.

Statistical Analysis Results were expressed as mean±SD. Statistical differences between means were determined using one-way ANOVA followed by two-tail Student's t-test when appropriate with the software SPSS 13.0 for windows. P<0.05 was considered statistically significant.

RESULTS

Filtering Bleb Area The area of filtering bleb in both groups decreased with the time, respectively (Figure 1). Comparing with day 1 postoperatively, the control group reduced gradually since 3 days after GFS (P<0.05), while the antibody group 5 days after GFS (P<0.05). The results indicate the earlier filtering bleb fibrosis of control group comparing with the antibody group, and CTGF antibody may delay the fibrosis occurring. On the 1,3 and 5day after the operation, the bleb area of two groups had no significant difference (P>0.05), however on the day 7, 10 and 14 postoperatively, the antibody group filtering bleb area was greater than the control group, with a significant difference (P<0.01, P<0.05), and the results presented that since day 7after GFS, the antibody group bleb scarring much less than the control group, suggesting that CTGF antibody can prevent the scarring of filtering bleb.

IOP The IOP of both groups decreased after the glaucoma filtration surgery with a significance difference (P<0.01, Figure 2). The IOP of both groups increased with the time. Comparing with the day 1 postoperatively, on the day 7, 10 and 14, the difference between the two groups was significant (P<0.01, P<0.05), which presents the surgery can reduce the IOP and the conjunctiva tissue proliferation may exist in the surgical area. Either before the operation or day 1, 3 and 5 after, there was no significant difference of IOP between the two groups (P>0.05). In the day 7, 10 and 14 postoperatively, the IOP were lower in antibody group (P<0.01). The result suggested the injection of CTGF antibody subconjunctively during the operation and 5 days
after could slow down the rebound of IOP, maintained relatively lower pressure and therefore functioned as anti-scarring reagent.

DISCUSSION
It has been proved by scores of studies that TGF-β serves as an important cytokines for filtering bleb scarring. Meyer et al. studied the cell type specific localization of TGF-β receptors in sections of normal conjunctiva and scarred filtering blebs to further elucidate its possible role in filtering bleb scarring after glaucoma surgery in human eyes in vivo. It is shown that filtering bleb scarring is associated with an abundant expression of TGF-beta receptors in activated fibroblasts. These data support the concept of targeting TGF-β signaling to prevent scar formation after filtering glaucoma surgery. CTGF exists in connective tissue as a cytokine activated selectively by TGF-β. CTGF could mimic many biological behaviors lead to scarring been proved by studies, such as fibroblast proliferation, migration and production of ECM. According to the study of Wu et al., during the healing of cornea trauma of rabbits, TGF may up regulate the expression of CTGF, as a downstream factor of TGF, CTGF mainly regulates the synthesis of collagen tissues and determines the amount of connective tissue produced and the later formation of scar. Seher et al. study specified the importance of CTGF for primary tenon fibroblast function. The RNA expression profile yields new insights into the relevance of CTGF in influencing biologic processes like wound healing, inflammation, proliferation, and extracellular matrix remodelling in vitro via transcriptional regulation of specific genes. The results suggest that CTGF potentially acts as a modulating factor in inflammatory and wound healing response in fibroblasts of the human eye. Yuan et al. investigated the role of CTGF after trabeculectomy associated with wound healing and to identify the role of CTGF in this process, and the results suggest that overexpression of CTGF in the blebs after trabeculectomy demonstrates that CTGF may play an important role in the process of wound healing. Furthermore, ocular hypertension may be involved in the upregulation of CTGF expression. All the studies mentioned above explain TGF operates scarring effect through CTGF, blocking CTGF may restrain the scarring of filtering bleb. Sherwood et al. created the glaucoma filtration surgery mold with drainage tube implantation, comparing with those injected with balanced salt group whose filtering bleb survive 17 days, the group injected CTGF antibody subconjunctively lasted 26 days, which indicated that CTGF antibody could prolong the survival time of filtering bleb as anti-scarring reagent. However, this research included neither the observation of IOP variation postoperatively, nor the change of area of filtering bleb. According to our study, CTGF antibody injected subconjunctively exceeds the group with PBS in the filtering bleb area and the IOP of the former group is lower, suggests the anti-scarring of CTGF antibody and coincides with Sherwood study. CTGF antibody may become a new anti-scarring medicine for filtering bleb and requires further study.

The time for CTGF antibody injection is critical. Esson et al. reported the markedly higher expression of TGF-B and CTGF in filtering bleb post-trabeculectomy with ELISA and immunohistochemical study. The most obvious immunofluorescence imaging appeared in day 5 and 7 postoperatively. CTGF level decreased in the day 1 after the operation, increased tremendously in day 3 and reached the peak in the day 5, since day 7, it started to decline. Both returned to normal baseline in day 21 postoperatively. The level of CTGF expression after trabeculectomy emerges a "single-hump" shape, theoretically, blocking CTGF in day 3 and 5 postoperatively can counter the scarring of filtering bleb. We injected CTGF antibody subconjunctively by the end of the surgery. Both the filtering bleb area and IOP of the control group exceeded the antibody group slightly in day 1 postoperative. The bleb area of the antibody group is larger than the control in day 3 and 5 postoperative, whereas IOP is lower and no significant difference, which indicates the CTGF antibody has not yet acted to refrain the scarring. The level of CTGF expression in filtering bleb after the filtration surgery has not been reported. If CTGF is in low level at early time after operation, the CTGF antibody will be weak in anti-scarring, therefore, the insignificant difference between two groups in the day 1, 3 and 5 postoperatively should not be interpreted as no anti-scarring effect for the CTGF antibody. On the day 5 after the surgery, CTGF antibody injection was repeated after measuring the filtering bleb area and IOP. On the day 7 after the surgery, the bleb area was larger while the IOP was lower in antibody group, and there was a significant difference, which exhibits the CTGF antibody injected on day 5 performed anti-scarring effects. Even afterwards, no more CTGF antibody was injected, the filtering bleb area in antibody group were all larger than that of the other group. IOP has the same results on day 10 and 14, and the difference has a significance. This indicates the blockage of CTGF in its peak time on day 5 could have a long term effect in anti-scarring.

The site for injection is also very important. The study of different concentration gradient and dose gradient of CTGF antibody injection to subconjunctiva may disclose the optimum condition for anti-scarring, if possible, which will be significant in its clinical application. In our experiment,
50mg/L CTGF antibody 0.1mL was injected subconjunctivally in the nasal side of superior rectus muscle and acted to refrain scarring in the filtering bleb area, which indicates the method we apply is effective. All in all, in the peak expression time of CTGF in day 5 after the surgery, subconjunctive injection of CTGF antibody can conspicuously contradict bleb scarring and improve the success ratio of trabeculectomy surgery, and the details like the time spot, concentration, dose and site to inject CTGF antibody will require further study.

REFERENCES
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