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Concentration change of TGF- β 1 in aqueous humor of rabbits

Xiao-Yi Yu^{1,2}, Wei Wu¹, Xiao-He Lu^{1*}¹Department of Ophthalmology, Southern Medical University, Guangzhou 510282, Guangdong Province, China²Department of Ophthalmology, Wuyi Traditional Chinese Medicine Hospital, Jiangmen, Guangdong Province, China

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ABSTRACT

Objective: To observe the influence of the transforming growth factor β 1 (TGF- β 1) eye drops on rabbit aqueous humor TGF- β 1 concentration, and to analyze the best drug concentration. **Methods:** A total of 30 New Zealand white rabbits were randomly divided into 5 groups with 6 in each. Rabbits in control group had PBS eye drops, group A, B, C, D adopted TGF- β 1 eye drops at 0.5, 1.0, 2.0, 4.0 mg/L, respectively, 4 times a day. Aqueous humor of right eye was extracted 1 week after administration to detect concentration changes of TGF- β 1 by ELISA; rabbits in four groups adopted 2.0 mg/L eye drops to left eyes 4 times a day, 0.2 mL aqueous humor was extracted left eye at the scheduled time point 0, 30 min, 2 h, 4 h, 24 h for testing, the slit lamp was used to observe the cornea, chamber and lens. **Results:** No obvious pathological changes in conjunctiva, cornea, rabbit conjunctival, anterior chamber, and the lens was found. Concentration of TGF- β 1 in rabbit aqueous humor in C, D group was significantly higher than the control group ($P < 0.05$). **Conclusions:** TGF- β 1 eye drops at 2.0 mg/L, 4.0 mg/L can significantly increase concentration of TGF- β 1 in rabbit aqueous humor, with good ocular surface permeability.

1. Introduction

Transforming growth factor β (TGF- β) family, including three members: TGF- β 1, TGF- β 2, TGF- β 3[1], is made up of inactive peptide dimer of about 25 kDa molecular weight, secreted by small complex[2]. TGF- β is widely expressed in mammalian body. Studies have found that Latent TGF- β binding proteins (LTBPs) can mediate TGF- β and cornea in combination with the specific extracellular matrix, so as to improve the biological activities of TGF- β [3–5]. This study aimed to observe effect of inhibitors TGF- β 1 eye drops on concentration of growth factor β 1 in aqueous humor mass. The results are as follows.

2. Materials and methods

2.1. Experimental animals

A total of 30 adult New Zealand white rabbits were

selected, provided by the Laboratory Animal Center, Southern Medical University. 34 and 37 weeks, weight 3–4 kg, male and female unlimited, disposing experiments animals in accordance with “Guidance on Treating Experimental Animals”[6]. Experimental design was randomized controlled animal experiment, laboratory biosafety level of BSL-3.

2.2. Instruments and reagents

Model 680 enzyme standard instrument, Model 1575 washing machine (BIO-RAD, USA); Eppendorf R5415 centrifuges at low temperature; DNP-9082 type thermostatic incubator (Shanghai jing hong laboratory equipment co., LTD.); Dry powder of growth factors β 1 (SANTA company, USA); Rabbit TGF- β 1 ELISA Kit (wuhan booster production).

2.3. Methods

A total of 30 New Zealand white rabbits were randomly divided into 5 groups with 6 in each group. Rabbits in control group had PBS eye drops, group A, B, C, D adopted TGF- β 1 eye drops at 0.5, 1.0, 2.0, 4.0 mg/L, respectively, 4 times a day. Aqueous humor of right eye was extracted 1 week

*Corresponding author: Xiao-He Lu, PhD, Department of Ophthalmology, Southern Medical University, Guangzhou 510282, Guangdong Province, China.

Tel: 15002000613

E-mail: yuxiaoyi1107@163.com

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after administration to detect concentration changes of TGF- β 1 by ELISA; rabbits in four groups adopted 2.0 mg/L eye drops to left eyes 4 times a day, 0.2 mL aqueous humor was extracted left eye at the scheduled time point 0, 30 min, 2 h, 4 h, 24 h for testing, the slit lamp was used to observe the cornea, chamber and lens.

2.4. Indexes observation

Eye changes of 5 groups were observed under slit lamp, and the ELISA method was used to test drug concentration in aqueous humor of the right eye and concentration of TGF- β 1 in left eye aqueous humor at different time points. Aqueous humor was centrifugated at low speed for 10 min, the supernatant was stored in EP tube at -20°C . The prepared samples was added into standard rabbit TGF- β 1 at 37°C for 90 min; They were washed 2 times, biotin rabbit TGF- β 1 antibody working liquid was added for 60 min at 37°C . Then ABC working liquid was added at 37°C for 30 min, TMB color liquid was added at 37°C . After adding TMB terminated liquid, 450 nm standard enzyme instrument was used immediately to measure OD value by standard curve method.

2.5. Statistical analysis

SPSS13.0 statistical software was used to analyze experimental data, then it was analyzed by *t* test. All data were expressed as $\bar{x}\pm s$, $P < 0.05$ was considered as statistically significant difference.

3. Results

3.1. Slit lamp observations

No conjunctival secretions, conjunctival congestion, edema, corneal epithelial defect or calm occurred. Figure 1 showed that TGF- β 1 eye drops was non-toxic to cornea and conjunctival.

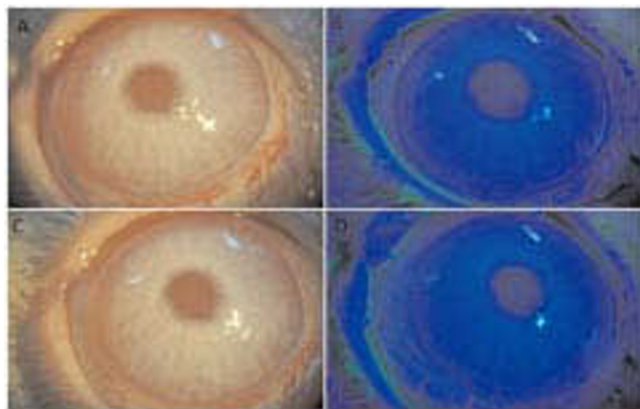


Figure 1. Slit lamp observations of TGF- β 1 group.

A: normal cornea appearance before medication; B: rabbit normal corneal fluorescein staining appearance before medication; C: corneal appearance after a week; D: corneal fluorescein staining appearance after a week medication.

3.2. Concentration change of TGF- β 1 in aqueous humor

Concentration of TGF- β 1 was (0.98 ± 0.26) ng/mL in control group, (1.06 ± 0.03) ng/mL in A group, (1.09 ± 0.15) ng/mL in B group, (2.36 ± 0.23) ng/mL in C group, and (2.44 ± 0.24) ng/mL in D group. There was no significant difference between A, B group and the control group ($P > 0.05$); the concentration in C, D groups was significantly higher than control ($P < 0.05$); differences between group C and D was not statistical significant. Concentration of TGF- β 1 in A, B, C, D was increased with elevated concentrations of eye drops (Figure 2).

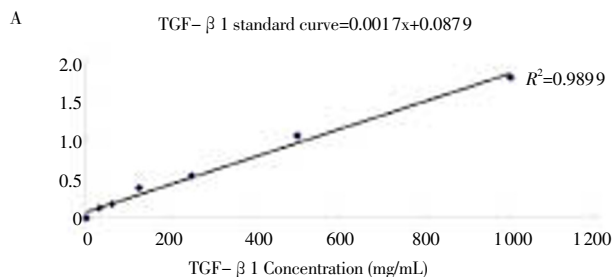


Figure 2. Standard curve method of OD value.

3.3. Concentration changes of TGF- β 1 in rabbit aqueous humor at different time points

After administration of TGF- β 1 eye droplet, TGF- β 1 was significantly increased within 0-2 h in rabbit aqueous humor, and reached the peak 1 h later. TGF- β was decreased after 2 h, and the decrease was stable, showing that normal rabbit aqueous humor contained certain TGF- β 1 (Figure 3).

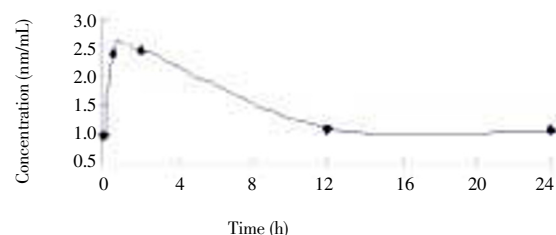


Figure 3. Concentration changes of TGF- β 1 in rabbit aqueous humor at different time points.

4. Discussion

At present, the role of TGF- β in corneal injury repair has been generally recognized. Expression of TGF- β 1 is strengthened in the cornea injury repair process[7-10]. Studies have found that[12,13], TGF- β 1 can significantly promote the corneal stromal hyperplasia, stimulate the corneal epithelium, stroma and directional migration of endothelial cells, and in the cornea injury repair reaction, TGF- β 1 content enriched significantly[15,16]. TGF- β 1 can promote corneal injury repair with epidermal growth factor, and plays an important role in early healing of eye incision, and postoperative corneal damage. Other research

has shown that^[17], TGF- β 1 can promote the reconstruction of the corneal damage matrix structure repair, reduce scar under corneal epithelium. Through inhibition of matrix metalloproteinases (MMPs) and activation of tissue inhibitors of MMPs (TIMPs) to adjust the corneal stroma reconstruction, which can reduce corneal epithelium scar. After photorefractive keratectomy, TGF- β 1 can obviously inhibit scar tissue hyperplasia and occurrence of corneal fibroblasts corneal epithelium under cloudy (Haze)^[18].

In recent years, studies have shown that^[19], TGF- β 1 has strong anti-inflammatory and immune suppression effect, can adjust multiple links section of the immune response. It has a good curative effect on the corneal transplantation immune rejection, and can promote healing of corneal. It can also suppress allogeneic corneal transplantation immune rejection and prolong corneal graft survival, curative effect is superior to corticosteroids and cyclosporine A^[20]. It can also prevent the corrosion process of the corneal ulcer, improve the healing of corneal surface and reepithelization, obviously increase the cure rate of the single blister late viral stromal inflammatory lesions, and shorten the course of the disease^[21,22]. The experimental results showed that the concentration of 2.0 mg/L and 4 mg/L TGF- β 1 eye drops can achieve effective concentration in the anterior chamber. Hence dose 2.0 mg/L should be given priority. And after a week, bulbar conjunctiva, conjunctiva, cornea, anterior chamber and the lens in rabbit eyes no obvious pathological changes were observed under slit-lamp, showing that TGF- β 1 eye drops is diagonal conjunctival non-toxic, security good, suitable for ophthalmology clinical application.

The results showed that 2.0 mg/L, 4.0 mg/L of TGF- β 1 eye drops can significantly increase concentration of TGF- β 1 in rabbit aqueous humor, with good ocular surface permeability.

Conflict of interest statement

We declare that we have no conflict of interest.

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