·Basic Research·

Influence and mechanism of He-Ne laser on scar formation of filtration canal after trabeculectomy in rabbit

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Abstract

• AIM: To investigate the influence of He-Ne laser on connective tissue growth factor (CTGF) expression and collagen formation of fibroblast in filtration site after trabeculectomy in rabbit, and to discuss the mechanism for preventing scar formation with He-Ne laser *in viva*

• METHODS: The upper nasal limbus area next to the upper rectus muscle in right eyes received 10 minutes He-Ne laser irradiation (200mW/cm^2) every day for three days, the left eyes served as control. Twenty-four hours after the last irradiation, both eyes of the rabbits were took trabeculectomy surgery. The expressions of CTGF in the filtration area were tested on the 7th, 14th and 28th day after surgery and collagen density was tested on the 14th and 28th day after surgery. Each of the time point had 7 rabbits.

• RESULTS: The expression of CTGF was lower than that of the control group's on the 7th and 14th day after trabeculectomy surgery (P=0.01, P=0.005). When examined on the 14th and 28th day, the collagen density of irradiation group were significantly lower than that of the control group's (P=0.013, P=0.01).

• CONCLUSION: Pretreating the filtration area with 200mW/cm^2 He-Ne laser may be helpful in preventing scar formation after trabeculectomy in rabbit, possibly due to downregulation of the expression of CTGF and collagen synthesis in fibroblasts. He-Ne laser may be developed into a new scar preventing method in filtration surgery.

• KEYWORDS: He-Ne laser; filtration surgery; CTGF; collagen

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INTRODUCTION

Scar formation of the filtration channel is one of the most important causes to the failure of trabeculectomy. It is also the most intractable problem of the anti-glaucoma surgery. How to make a permanent channel for the outflow of aqueous humor is the challenge all ophthalmologist confronted with.

Mitomycin and 5-Fluorouracil are widespread used in clinical nowadays, and to some extent they can inhibit the excessive proliferation of fibroblast hypo-conjunctiva after the filtration surgery. However, these drugs also can cause some sever complications such as long time post surgery hypotension, filtration bubble leakage, endophthalmitis and *etc* [1-4]. So the discovery of new anti-scarring methods is very important to filtration surgery.

He-Ne laser can inhibit the collagen formation of fibroblast *in vitro*, reduce the dermatic scar effectively and prevent the scar formation of skin. It is widely used in cosmetology, and it is safe in effect dosage [5,6]. He-Ne laser may have great clinical significance in prevention of scar formation after the filtration surgery. This research use He-Ne laser on rabbit ocular, observe the scar formation after the filtration surgery, and discover the mechanism of He-Ne laser impact on the wound healing and scar formation.

MATERIALS AND METHODS

Materials

Experimental animal Twenty-one healthy sanitation grade New Zealand albino rabbits, weight about 2kg, no gender limit, provided by the Experimental Animal Center of Tongji Medical College.

Instruments and reagents The He-Ne laser machine used in this study was manufactured by Wuhan Nation

Opto-electricity Laboratory max power density is 300mW/cm^2, light spot diameter is 4mm. Rabbit anti-CTGF multi-clone antibody (Wuhan Boster); SABC kit (Wuhan Boster); DAB kit (Wuhan Boster) and Masson staining kit (Wuhan Guge).

Methods

Laser irradiation Site: the upper nasal limbus area next to the upper rectus muscle; diameter: 4mm; time: 10 minutes×3 days.

Surgery method Rabbits were intravenous anesthetized with 30g/L Pentobarbital, conjunctival sac were washed by 3g/L FPA. Trabeculectomy: made a conjunctival flap based on fornix zone just next to the upper rectus muscle, and a 3mm ×3mm scleral flap (2/3 sclera thickness). Excised 1.5mm×× 1mm trabecula tissue in the corneoscleral limbus. Cut a 1.5mm ×1.5mm iris periphery hole. Reset the sclera flap, sutured the sclera flap and bulbar conjunctiva.

Animal grouping Twenty-one rabbits were randomly divided into three groups, representing the 7th, 14th and 28th day after surgery. The right eyes were received laser irradiation, the left eyes served as control. Seven eyes each group at each time point.

Observation and sample preparation We observed the filtration bubble after surgery. Rabbits were executed on the 7th, 14th and 28th day after surgery, took the tissues of surgery site(the whole layer),fixed them with 40g/L paraformaldehyde solution for 24 hours, then paraffin imbedded and cut into slices.

Histopathological and Immunohistochemistry Examin – ation

Masson´s staining We observed the proliferation of collagen in the filtration canal. Result criterion: the collagen was blue stained while the muscle fiber and cellulose were red stained. The slice was put under the microscope in the same light intensity to observe the blue stained area and image analysis software was used to calculate average luminosity.

CTGF immunohistochemistry staining We used the SABC kit to mark the antigen (CTGF) and observe after DAB staining. Result criterion: the CTGF staining positive cell were those with brown or dark brown kytoplasm. Five high power lens (×400) visual field were chosen randomly in every slice, we counted the positive and whole number of fibroblast, and calculated the average positive cell percentage.

Statistical Analysis The SPSS (16.0) software was used. *T-test* was used to compare the expression of CTGF and collagen density groups. $P<0.05$ was assumed the sign for diversity had statistical significance.

Table 1 He-Ne laser impact on the CTGF expression (%) of fibroblast in filtration site (mean±SD, $n=7$)

Groups	the 7th day	the 14th day	the 28th day
Control	24.57±3.95	12.67±3.13	4.67±1.71
Laser	17.14±5.01	7.43±2.51	4.76±1.50
T	3.078	3.458	-0.110
P	0.01	0.005	0.915

Table 2 He-Ne laser impact on the collagen expression (%) in the filtration site (mean±SD, $n=7$)

Groups	the 14th day	the 28th day
Control	10.46±2.40	21.30±4.69
Laser	7.78±0.48	15.69±1.39
P	0.013	0.01

RESULTS

When observed on the 1st, 7th and 14th day after surgery, hyperemia was much lesser and filtration bubble was much more obvious in laser group than those of control group.

The expression of connective tissue growth factor (CTGF) in the filtration site was notable on the 7th day, lesser on the 14th day, and a very small amount on the 28th day (Figure 1). The expression of laser group was much lesser than that of control group on the 7th and 14th day ($t=3.078$, 3.458; $P=0.01$, 0.005), and the expression diversity between the two groups on the 28th day had no statistical significance ($t=-0.110$, $P=0.915$) (Table 1). Masson's staining and image analysis showed that collagen expression was obviously lesser in laser group on the 14th and 28th day (Figure 2) ($t=2.914$, 3.032; $P=0.013$, 0.01) (Table 2).

DISCUSSION

How to sustain a functional filtration bubble after surgery is very important, suppress the scar formation of filtration canal effectively is the key point for the surgery. The scar formation of filtration canal is induced by the inflammatory factors result in tissue damage during the surgery. The factors stimulate the inflammatory reactions and the proliferation and shifting of fibroblast. Fibroblast is functional active fibro cell, can synthesis and secrete collagen, induce the massive proliferation of extracellular matrix as collagen fiber and elastic fiber, cause the scar formation of filtration canal and subconjunctiva tissue fibration and finally result in stenosis even shut down of the filtration canal. So the research is focused on the fibroblast.

He-Ne laser machine is the first manufactured continuous emission gas laser, and is the most reliable one. It is widely used because of its portability. The working substances are helium and neon, and neon is the working gas. The laser is produced by neon atom, and mainly stimulates three wavelength, 632.8nm, 1.15μm and 3.39μm. The output power of laser is low, 0.1-100mW generally. Organism exposed into He-Ne laser will not result in nonreversible

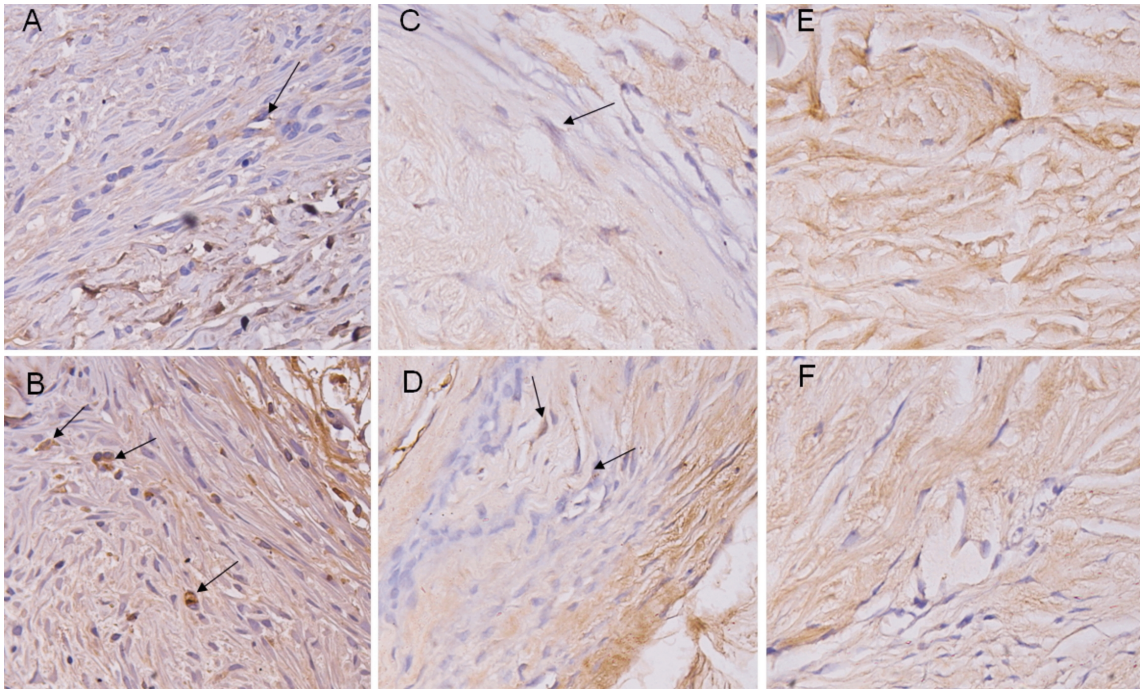

Figure 1 CTGF expression of fibroblast in the filtration site (×400, arrow pointed are positive cells) A: Laser group on the 7th day after surgery: a small amount of CTGF positive cells in filtration site; B: Control group on the 7th day after surgery: a lot of positive cells in filtration site, and they are stained darker; C: Laser group on the 14th day after surgery: few CTGF positive cells in filtration site and stained lighter compare to the 7th-day group; D: Control group on the 14th day after surgery: a small amount of CTGF positive cells in filtration site, and stained lighter compare to the 7th-day group; E and F are laser and control group on the 28th day after surgery: a extremely small amount of CTGF positive cells in filtration site

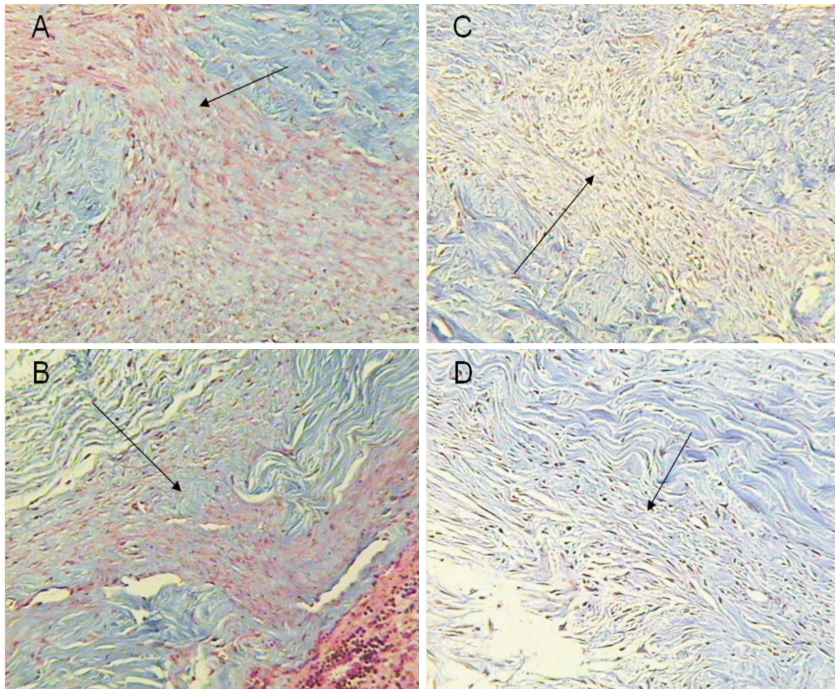

Figure 2 Arrow pointed is the filtration site, and blue stained tissue is collagen fiber (×200) A and B are laser and control group on the 14th day; C and D are laser and control group on the 28th day, and the collagen expression is higher in laser group

tissue damage, so it is safe[7-9].

According to the research, He-Ne laser impact on organism is related to its power and energy density. Low dose He-Ne laser radiation promotes the fibroblast proliferation and collagen synthesis, and cause wound healing. High dose He-Ne laser radiation inhibits the fibroblast proliferation and promotes apoptosis[10-14]. This research uses high level dose of He-Ne laser to study its influence on the scar formation of the filtration canal.

Connective Tissue Growth Factor (CTGF) is new

discovered fibrosis factor in the 1990s, is a polypeptide rich in cysteine, and a member of CNN family. CTGF is widely expressed in many human tissues and cells, such as heart, brain, lung, kidney, placenta, pancreas and connective tissue. Its main functions are: promote cell proliferation and collagen synthesis, induce cell adhesion and chemotaxis, and facilitate blood vessel and granulation tissue formation. CTGF is none or extremely low expression in normal tissue[15]. But CTGF is closely related to some hyperplastic and fibrosis diseases in pathologic status. Many researches approved that CTGF expression is positive correlation to the degree of fibrosis [16]. As the important substance of ECM, collagen is secreted by fibroblast, and its degree of proliferation can reveal the degree of scar formation.

The research shows that the fibroblast proliferation and collagen synthesis are most active on the 7th day after surgery, the activity went down on the 14th day, and went normal on the 28th day. All above are accord with the post surgery inflammatory process, is the same as the literature report [17-19]. The research result points out that the pretreatment with the He-Ne laser can improve the success rate of the filtration surgery significantly, represented by the good condition of the filtration bubble. This could be the result of He-Ne laser downgrade the fibroblast CTGF expression and the activity of fibroblast proliferation and collagen synthesis, reduce the inflammatory reaction, and scar formation of filtration canal.

The mechanism of He-Ne laser inhibit the proliferation of cell is not completely clear. Its impact on the proliferation and apoptosis of cell, and the safety of using He-Ne laser on ocular need more study.

In conclusion, the He-Ne laser with 200mW/cm^2 power density can reduce the scar formation of rabbit ocular after the filtration surgery; it could be related to the downgrading of fibroblast CTGF expression and collagen synthesis. It is possible the He-Ne laser could be a brand-new way to improve the success rate of filtration surgery.

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