

LMWH inhibits anterior chamber inflammation after extra capsular lens extraction through down regulation of bFGF content in aqueous humor

Jian-Ming Wang, Lei Xiong, Quan-Chen Xiong, Ya-Zhi Fan

Foundation item: Science and Technology Research Foundation of Shaanxi Province, China [No.2008K14-07(2)] Department of Ophthalmology, the Second Affiliated Hospital, Medical School of Xi'an Jiaotong University, Xi'an 710004, Shaanxi Province, China

Correspondence to: Jian-Ming Wang. Department of Ophthalmology, the Second Affiliated Hospital, Medical School of Xi'an Jiaotong University, Xi'an 710004, Shaanxi Province, China. xajdwjm@163.com

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Abstract

• **AIM:** To observe the changes of basic fibroblast growth factor (bFGF) content in anterior chamber before and after extra capsular lens extraction for investigating the mechanism of low molecular weight heparin (LMWH) inhibiting anterior chamber inflammation.

• **METHODS:** Eighty-four rabbits were randomly divided into control and experimental group, 42 rabbits in each group. Extra capsular lens extraction was done on unilateral eye in each rabbit. LMWH was perfused into anterior chamber by the concentration of 50U/mL at the end of operation in experimental group. The degrees of corneal edema, aqueous flare and fibrin were evaluated with slit lamp microscope on postoperative day 1, 3, 6, 15, 30, 45 and 60, respectively. Six eyes of each group were at each time point. Contents of bFGF in aqueous humor were determined by ELISA after animals were killed. Another six eyes were used for determining the base line level of bFGF in aqueous humor.

• **RESULTS:** The degrees of corneal edema, aqueous flare and fibrin in experimental group were significantly lighter than those in control group ($P < 0.01$) on postoperative day 1, 3 and 6, respectively. No difference was showed between the two groups at other point time. Contents of bFGF in aqueous humor increased at the same time. bFGF content was reached peak on postoperative day 1 in experimental group, while on postoperative day 6 in control group. Contents of bFGF in the two groups declined slowly after reaching peak. The bFGF content in control group were significantly higher than that in experimental group 1-30 days after surgery ($P < 0.05$). No significant differences were shown between the two

groups on postoperative day 45 and 60, respectively.

• **CONCLUSION:** Perfusion with LMWH by the concentration of 50U/mL can significantly reduce anterior chamber inflammation after extra capsular lens extraction in rabbits, which may be related to down regulation of bFGF content in aqueous humor.

• **KEYWORDS:** low molecular weight heparin; inflammation; aqueous humor; basic fibroblast growth factor; extracapsular lens extraction

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INTRODUCTION

Low molecular weight heparin (LMWH) is 4000Da-6500Da of low molecular weight fractions, depolymerized from heparin. LMWH is a traditional anticoagulant drug with curative effect. It has been clinically applied for many years with fewer side effects. Its antithrombotic effect is superior to heparin, and anticoagulant effect is less than heparin. It has high bioavailability, long half-life *in vivo*, less bleeding tendency, and good oral absorption. Due to its effectiveness and safety superior to unfractionated heparin, LMWH has become research hotspot. In recent years, it had been found that LMWH also possesses anti-inflammatory, antiproliferative and anti-tumor effects^[1,2]. It has been applied to clinical ophthalmology, and the results showed that LMWH could relieve the eye fibrous exudation, reduce proliferation, reduce corneal edema, and reduce posterior capsule opacification^[3,4]. But these are limited to clinical observation, no relevantly further mechanism research. Basic fibroblast growth factor (bFGF) is an important inflammatory factor and it involved in intraocular inflammation and proliferation reaction process^[5-7]. LMWH could inhibit bFGF induced cell proliferation in cultured canine arterial smooth muscle cell^[8]. It was shown that LMWH inhibit cell proliferation effect

may be related to the inhibition of bFGF. It has not been reported the effects of bFGF on inhibition of intraocular inflammation after cataract extraction following LMWH application. Therefore, we observed the inhibited effects of anterior chamber inflammation reaction after the cataract operation combined with LMWH, and determined real content of bFGF in aqueous humor at different time after operation. The effect mechanism of LMWH on anterior chamber inflammation reaction after cataract operation will be investigated.

MATERIALS AND METHODS

Materials Ninety eyes were from 90 healthy New Zealand white rabbits with male and female, and the body weight of each animal about 2kg. All rabbits were from the School of Medicine of Xi'an Jiaotong University experimental animal center. No abnormality was found in all eyes with slit-lamp examination before operation. Sumianxin injection for Anesthesia was made by Agricultural University Military Veterinary Institute. LMWH injection (the trade name: Fraxiparine) was made by the French pharmaceutical company GlaxoSmithKline. ELISA reagent kit for determination of the content of bFGF in aqueous humor was made by the United States of America ADL Company.

Methods Six of ninety rabbits were randomly selected as blank control group under local anesthesia to extract aqueous humor of monocular and save for testing. The remaining 84 rabbits were randomly divided into control group and experimental group (42 eyes each group), and monocular transparent lens extracapsular cataract extraction was made in each rabbit under general anesthesia. The operation mode in two groups was the same, with ophthalmic balanced salt as intraoperative infusion liquid. Eyes of the experimental group were perfused with 10mL 50U/mL LMWH into anterior chamber about 1-2 minutes after closing the scleral incision, but eyes in control group were not using LMWH. All the operations were performed by the same surgeon. The degrees of corneal edema, turbid aqueous and anterior chamber fibrin exudation were as the anterior chamber inflammation observed indexes.

The degrees of corneal edema, turbid aqueous and anterior chamber fibrin exudation were observed by slit lamp and grade evaluation in 6 rabbits on day 1, 3, 6, 15, 30, 45 and 60 in each group. After slit lamp observation, the animals were killed and aqueous humor was extracted for determining of the content of bFGF using ELISA method. Anterior chamber puncture was made at corneal limbus before aqueous extraction using 23G needle in 1 minute. 0.3mL-0.4mL aqueous humor was extracted and placed in a sterilized centrifuge tube, then immediately stored at -80°C. Before determined bFGF content, cryopreserved aqueous humor should be melt at room temperature.

Corneal edema degree classification was according to Nagahara *et al*^[9]'s standard. Grade 0 for corneal edema only limited at operation incision area; Grade 1 for slight corneal edema, and the iris texture can be seen; Grade 2 for moderate edema, and the iris texture fuzzy visible; Grade 3 for corneal complete opacification, and the iris cannot be seen.

Turbidity degree classification of aqueous humor was according to Wu *et al*^[10]'s standard. Grade 0 means 5 suspended particulate visible under strong beam of light, Tyndall syndrome positive; Grade 1 means 6-10 suspended particulate visible under strong beam of light, Tyndall syndrome positive; Grade 2 means 10 or more number of suspended particulate visible under strong beam of light, Tyndall syndrome were significantly positive; Grade 3 means significant pollution of aqueous humor, and suspended particulate uncountable, Tyndall syndrome strongly positive.

Fibrin exudation grading was according to Moon *et al*^[11]'s standard. Grade 0 means no fibrin exudation; Grade 1 means anterior chamber fibrin exudation is gossamer, or short fibers attached to the iris and pupil, but do not form across the bridge shape adhesion; Grade 2 means fibrin formation in thickness, covering less than 50% iris pupil surface; Grade 3 means fibrin exudation forming the whole film, or less than 1/2 area of the anterior chamber fiber clot; Grade 4 means fibrin clot area greater than 1/2 of anterior chamber.

Statistical Analysis Results of bFGF content in anterior chamber were expressed as mean±SD. Statistical differences of the data were determined with the software SPSS 13.0 for Windows. $P<0.05$ was considered statistically significant. Grade data were compared with the Mann-Whitney U -test. Measurement data required to perform the normal distribution test and the test of homogeneity of variance. After according with normal distribution and homogeneity of variance, data between the two groups at the same time point were compared using paired t test, and data among two groups at different time points compared with single factor analysis of variance.

RESULTS

Anterior Chamber Inflammation Anterior chamber inflammation gradually reduced on postoperative day 7-15 in both groups, and disappeared after 15 days. The degree of corneal edema, turbid aqueous and anterior chamber fibrin exudation were shown more lighter on day 3 in experimental group after operation compared to those control group, and the differences had statistically significant (all $P<0.01$, Table 1). It had no significant difference between the two groups 15 days after operation. The result showed that anterior chamber perfusion with LMWH which concentration is 50U/mL could reduce postoperative corneal edema, turbid aqueous and anterior chamber fibrin

Table 1 Anterior chamber inflammation 3 days after operation (n=36)

Degrees	Corneal edema		Turbid aqueous		Fibrin exudation	
	Control	Experimental ^b	Control	Experimental ^b	Control	Experimental ^b
0	5	20	6	33	3	33
1	10	16	9	3	11	2
2	16	0	15	0	15	1
3	4	0	6	0	7	0

^bP<0.01 vs control.

exudation. It indicated that LMWH reduced anterior chamber inflammation after operation and promoted disease recovery.

Aqueous BFGF Content Determination The aqueous bFGF content both in experimental group and control group increased day 1 after operation, and the value in control group was significantly higher than that in experimental group ($P<0.05$). Values in experimental group decreased 3 days after operation and had no difference compared to the baseline. Values in control group gradually increased up to peak on day 6 after operation, and returned to the preoperative level on day 45 after operation. There were significant differences between two groups from 3-30 days after operation ($P<0.01$) and no differences from 45 days after operation. It was shown that 50U/mL LMWH perfusion into anterior chamber significantly reduced aqueous bFGF content after lens extraction (Table 2).

DISCUSSION

Anterior chamber inflammation is the most common complication after cataract operation, and the clinical manifestations are corneal edema, turbid aqueous and fibrin exudation. Anterior chamber inflammation usually can recover soon after treatment. But in diabetes and endothelial corneal dystrophy and other related diseases patients, anterior chamber inflammation has serious harm. It may be caused bullous keratopathy, seclusion of pupil, and secondary glaucoma. Anterior chamber inflammation also plays an important role in promoting posterior capsular proliferation and after cataract occurrence. Blood aqueous barrier disruption is the main cause of anterior chamber inflammation after cataract extraction operation. The blood components enter the anterior chamber and are activated following blood aqueous barrier disruption. Then fibrin coagulation system will start up and produce pro-inflammatory cytokines such as peanuts four acid, prostaglandins, bFGF, IL-1, IL-6, NO, oxygen free radical. These pro-inflammatory cytokines in turn will aggravate the anterior chamber inflammation^[12].

Heparin is a kind of conventional anticoagulant drug. In recent years, it is been found that heparin has anti-inflammatory and anti-tumor effects. LMWH not only has the above characteristics, but also has a smaller hemorrhagic risk^[1,2]. Therefore, LMWH has been added into perfusion fluid in cataract extraction operation for

Table 2 Aqueous bFGF content determination ($\bar{x} \pm s$, ng/L, n=36)

Postoperative day	Control	Experimental
0	16.4±6.9	
1	148.7±45.2	51.5±35.3 ^a
3	179.7±51.1	30.9±26.4 ^b
6	254.3±43.7	12.9±7.7 ^b
15	226.0±36.9	21.2±8.3 ^b
30	130.4±39.4	13.1±7.2 ^b
45	35.5±23.2	16.2±7.7
60	34.3±26.9	18.3±7.0

^aP<0.05, ^bP<0.01 vs control.

suppressing postoperative anterior chamber inflammation. It is shown that LMWH can inhibit inflammation in anterior chamber and posterior capsular opacification after cataract surgery^[4]. In our research, all the observation parameter values of inflammation were significantly lower in experimental group than that in control group after the perfusion of LMWH into anterior chamber in cataract surgery. The result was similar to that of Wu *et al*^[10]s. And the result further illustrated inhibition effects of the postoperative inflammation following LMWH of anterior chamber perfusion.

BFGF is a group of proteins which molecular weight is 22kDa-34kDa. It exists in almost all animals *in vivo* and bFGF from different species have a high degree of homology sequence. BFGF is widely distributed in the eye, and plays an important role in wound healing process, cell migration and cell proliferation. Data had shown that, during the inflammatory process, bFGF promoted inflammatory factors produced, had chemotaxis of inflammatory cells and increased cell-cell adhesion, and bFGF content increased in a variety of inflammatory diseases. Therefore, bFGF as a kind of inflammatory factor, become a kind of indicators responding to inflammatory intuitively, accurately, quantitatively^[13]. Wallentin *et al*^[14] observed the dynamic changing process of bFGF in aqueous humor after cataract extraction, and found that the bFGF content value gradually rose at first and then gradually decreased, its high platform with a longer duration. The results suggested that postoperative anterior chamber inflammation was related to content increase of bFGF in anterior chamber. Changing process of bFGF in anterior chamber after operation in our

study of control group was similar to that of Wallentin *et al* [14]'s results. But the dynamic changing process of bFGF in aqueous humor has not been reported following LMWH perfusion in cataract extraction. The aqueous bFGF peak in experimental group following LMWH anterior chamber perfusion was lower than that in control group, and only 1/5 of values in control group, and quickly recovered to the baseline level 6 days later in experimental group. Results indicated that LMWH anterior chamber perfusion reduced aqueous bFGF generation and alleviated anterior chamber inflammation. Changes trends of anterior chamber inflammation and bFGF content were same in both two groups, and this indicated that LMWH inhibited anterior chamber inflammation after lens extracapsular cataract extraction *via* down-regulation of aqueous bFGF content.

BFGF is mainly stored in heparin sulfate proteoglycans in the extracellular matrix. The main causes of bFGF content in aqueous humor reducing after LMWH anterior chamber perfusion may be the following: 1) LMWH can compete for binding to heparin enzyme, thereby reduce the heparin sulfate glycoprotein degradation, and reduce bFGF releasing; 2) LMWH can compete for binding to a variety of cytokines and chemokines, reduce stimulating the tissues and cells, thereby reduce the bFGF generation. Anterior chamber inflammation results from multiple factors, and its pathogenesis is very complex, involving multiple cytokine involvement. At present, there are many methods to inhibit anterior chamber inflammation, such as instillation and subconjunctival injection of anti-inflammatory drug. The administration of LMWH anterior chamber perfusion can inhibit anterior chamber inflammation at early stage after cataract surgery, reduce aqueous inflammatory factor, and may become a new method of inhibiting anterior chamber inflammation.

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