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The Measurement of Fibronectin Concentrations in Human Aqueous Humor

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The concentrations of fibronectin in aqueous humor, measured by ELISA which was developed to detect fibronectin, ranged from 5 ng/ml to 100 ng/ml. Aqueous humor was aspirated from human eyes with cataracts and glaucomas using a 26 gauge needle through the peripheral cornea before making the limbal incision into the anterior chamber during surgery. The results of the study show that the average concentration and standard deviation of fibronectin was 0.136 ± 0.192 μg/ml in cataract eyes, and 0.962 ± 0.918 μg/ml in glaucoma eyes respectively. There was a statistically significant difference between both groups (p = 0.000). However, no significant differences according to age and sex were noted. There was no influence due to preoperative intravenous mannitol injection on fibronectin concentration. The source of aqueous fibronectin is still not clearly known and the mechanism of the higher concentration of fibronectin in glaucoma has not been clearly disclosed, however it is thought that normally present fibronectin is accumulated in the anterior chamber because it can not pass the aqueous outflow pathway, or that fibronectin production may be increased in glaucoma.

Key words: cataract, ELISA, fibronectin, glaucoma, human aqueous humor

INTRODUCTION

Fibronectin is a disulfide-bonded glycoprotein with a subunit molecular weight between 200,000 and 250,000. Plasma fibronectin or "cold-insoluble globulin" has been isolated from plasma, serum, and amniotic fluid. Cell surface fibronectin is synthesized in vitro by numerous diverse cell types, including fibroblasts, myoblasts, amniotic fluid cells, intestinal epithelial cells, vascular endothelial cells, and corneal endothelial cells.[1] It is well known that fibronectin is effective in the treatment of persistent corneal epithelial defects.[2,3,4,5,6,7,8] Endogenous sources of fibronectin in corneal wound healing have been the subjects of attention for numerous researchers. As reported until now, tears, aqueous humor, limbal blood vessels, retrocorneal fibrous membrane, trabecular meshwork cells and the cornea itself were found to be the sources of fibronectin. The cornea itself has been suggested as the major source of fibronectin in corneal wound healing. However, it is uncertain which type of corneal cells mainly produce fibronectin in corneal wound healing.[9]

In 1978, Zetter[10] reported that corneal endothelial cells produced fibronectin in culture. In 1983 Scott,[11] and again in 1985, Baum and

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Hsieh[12] reported fibronectin mediated attachment of corneal endothelial cells and enhanced their growth in culture. In 1982, Reid *et al*[13] isolated fibronectin in bovine aqueous humor. Since the anterior chamber of the eye is lined with corneal endothelial cells and these cells produce fibronectin in culture, it seemed possible that fibronectin might be a component of aqueous humor. In our study, for the first time, we have isolated and measured the concentration of fibronectin in human aqueous humor.

MATERIALS AND METHODS

The human aqueous humor was aspirated from the anterior chamber through the peripheral cornea with a syringe and 26-gauge needle after placement of a superior rectus muscle bridle suture on cataract patients (48 eyes, 64.65 ± 14.57 years) and glaucoma patients (11 eyes, 49.82 ± 26.61 years) (Table 1). Cataract and glaucoma eyes with no history of diabetes or trauma were selected except for two cases of traumatic and hypermature cataract. Preoperative medications were administered through the instillation of 4% homatropine and 2.5% phenylephrine hydrochloride eye solution to cataract eyes for pupil dilation about 45 minutes prior to surgery, and systemic medication of dichlorphenamide (100 mg p.o.) and intravenous injection of mannitol (15%, 1.5 mg/Kg) to most patients for intraocular pressure control. The aspirated aqueous humors were immediately frozen under −20°C, then the concentration of fibronectin was measured by ELISA which can detect fibronectin ranged from 5 ng/ml to 100 ng/ml, and then the differences between cataracts and glaucomas, male and female, and mannitol (+) and (−) group were compared. Total protein in aqueous humor was measured by Lowry method and the fibronectin/protein ratio was compared between cataracts and glaucomas.

Table 1. Subjects

group	eyes	age (years)
cataracts	48	64.65 ± 14.57
glaucomas	11	49.82 ± 26.61
hypermature cataract	1	61
traumatic cataract	1	8
total	61	60.87 ± 19.44

Measurement of fibronectin concentration

Human plasma fibronectin is purified by affinity chromatography and then 100 μg fibronectin in 20 mM carbonate buffer, pH 9.6 containing 0.02% sodium azide is allowed to adsorb to the micro-titer well overnight at 4°C. After coating the plates, in a separate microtiter well, the aspirated human aqueous humor to be assayed or a standard sample of known fibronectin content are placed and are then mixed with an equal volume of 1 : 16000 dilution of goat anti-human fibronectin antibody to a final volume of 0.22 ml and incubated overnight at 4°C. The fibronectin coated plate is washed and 0.2 ml of the sample mixture and anti-fibronectin antibody is transferred to the coated plate and incubated for 30 minutes at room temperature. After washing the plate, 0.2 ml of 1 : 1000 dilution of antibody to the goat IgG made in rabbits and coupled to peroxidase is added and incubated for 90 minutes at room temperature. The plate is washed a final time and 0.2 ml of enzyme substrate is added and incubated for 1 hour at room temperature. The reaction is stopped with 50 μl of 4N H_2SO_4 and the product of the enzyme reaction is measured by ELISA reader. The enzyme substrate used was o-phenylenediamine dissolved in methanol at 10 mg/ml and diluted 1 : 100 into 0.03% H_2O_2 in distilled water. These substrates were freshly prepared (Fig. 1).

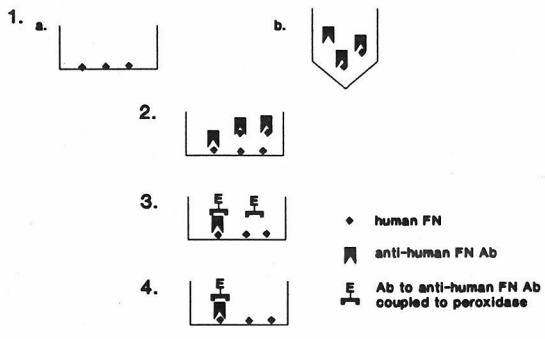

Fig. 1. Schematic procedure of an ELISA test for human aqueous fibronectin.

RESULTS

Human aqueous humor contained 0.136 ± 0.192 μg/ml of fibronectin in cataract eyes, and 0.962 ± 0.918 μg/ml in glaucoma eyes respectively. There was a statistically significant difference between both groups ($p = 0.000$). However, no significant differences according to age were noted. Fibronectin concentration in traumatic cataract and hypermature cataract was 1.44 ± 0.11 μg/ml i.e., as high as in glaucomas (Table 2, Fig. 2). In cataracts, fibronectin concentration was 0.088 ± 0.114 μg/ml in male and 0.158 ± 0.216 μg/ml in female. And in glaucomas, males had 0.757 ± 0.606 μg/ml of aqueous fibronectin and females had 1.007 ± 0.997 μg/ml. There tended to be a higher concentration of fibronectin in females than in males, but in each group, differences according to sex were not noted (Table 3). In cataract patients, subjects that had taken mannitol preperatively had 0.160 ± 0.215 μg/ml, and the others who had not taken mannitol had 0.073 ± 0.089 μg/ml of fibronectin. There were no differences between the mannitol (+) and mannitol (−) group ($p > 0.1$). In glaucoma patients, there was 0.769 ± 0.903 μg/ml of fibronectin in the mannitol (+) group, and 1.83 ± 0.24 μg/ml of fibronectin in the mannitol (−) group. There were also no significant differences (Table 4). Total protein was 0.56 ± 0.16 mg/ml in human cataract aqueous and 4.81 ± 5.77 mg/ml in glaucoma aqueous. Though the amount of protein in glaucomas was higher than in cataracts, there were no significant differences between both groups. The fibronectin /protein ratio was 0.024% in cataracts and 0.019% in glaucomas respectively (Table 5).

Table 2. Fibronectin concentrations in human aqueous humor

(μg/ml)

Age	Cataract	Glaucoma
0-19	—	1.830 ± 0.240
20-29	0.370 ± 0.377	—
30-39	0.026 ± 0.009	0.328
40-49	0.189 ± 0.248	—
50-59	0.128 ± 0.176	2.093 ± 1.283
60-69	0.126 ± 0.185	0.401 ± 0.271
total	0.136 ± 0.192	0.962 ± 0.918*

*$p < 0.001$

**hypermature cataract, corneal laceration; 1.440 ± 0.110 μg/ml

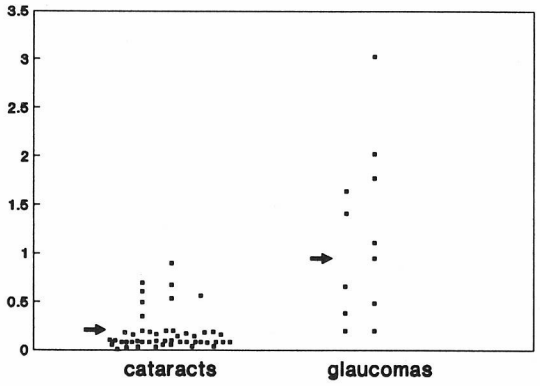

Fig. 2. Scattergram of fibronectin concentrations Arrow indicates mean concentration of fibronectin.

Table 3. Fibronectin concentrations in males and females

(μg/ml)

	Cataract	Glaucoma
Male	0.088 ± 0.114(15)	0.757 ± 0.606(2)
Female	0.158 ± 0.216(33)	1.007 ± 0.997(9)
total	0.136 ± 0.192(48)	0.962 ± 0.918(11)

(eyes)

Table 4. Fibronectin concentrations in mannitol (+ or −) groups

(μg/ml)

	Cataract	Glaucoma
Mannitol (+)	0.160 ± 0.215 (35)	0.769 ± 0.903 (9)
Mannitol (−)	0.073 ± 0.089 (13)	1.830 ± 0.240 (2)
total	0.136 ± 0.192 (48)	0.962 ± 0.918 (11)

(eyes)

Table 5. Comparison of human aqueous fibronectin and protein

	Cataract	Glaucoma
fibronectin (μg/ml)	0.136 ± 0.192	0.962 ± 0.918
protein (mg/ml)	0.56 ± 0.15	4.81 ± 5.77
FN/protein (%)	0.024	0.019

DISCUSSION

Comparing the human aqueous fibronectin concentration with the bovine aqueous fibronectin concentration in Reid's study[13] measured with gelatin Sepharose 4-B chromatography, the fibronectin concentration in bovine aqueous, 2.46 μg/ml, was higher than in human aqueous, 0.136 μg/ml in cataracts and 0.962 μg/ml in glaucomas. Fibronectin/protein ratio was another, in bovine aqueous 0.38%, and in human aqueous, 0.024% in cataracts and 0.019% in glaucomas (Table 6).

Table 6. Comparison of human and bovine aqueous humor

	Human		Bovine
	cataracts	glaucomas	
fibronectin (μg/ml)	0.136	0.962	2.46
protein (mg/ml)	0.560	4.814	0.65
FN/protein (%)	0.024	0.019	0.38

There are a number of possibilities for the source of fibronectin in aqueous humor. It has been demonstrated that in vitro, corneal endothelial cells produce fibronection.[10] It seems reasonable to postulate that these cells synthesize the fibronectin in vivo and release it into the aqueous humor. Another possibility is that aqueous fibronectin is produced by the ciliary body, since this is the source of aqueous humor. At the present time, however, the ciliary body has not been demonstrated to produce fibronectin in vitro. Another possible source is the lens capsule because it is a basement membrane and fibronectin is a component of basement membrane and considered to be an extracellular matrix glycoprotein. Heavy molecular-weight soluble proteins have been detected in the aqueous of patients with phacolytic glaucoma, mainly alpha and gamma crystallins that have a molecular weight greater than 150×10^6 daltons. The fourth possibility is that the trabecular meshwork cells can produce fibronectin which has been proved in vitro.[14] The presence of fibronectin in the aqueous humor offers exciting dimensions to the biology of corneal endothelial cells.

The blood-aqueous barrier normally limits the total protein content of the aqueous humor to less than one nineteenth (5.26%) of the plasma concentration. The protein composition of the aqueous differs markedly from that of the plasma, with lower molecular-weight proteins such as albumin and the beta-globulins being more prominent in the electrophoretic pattern of normal aqueous. The heavy molecular-weight proteins such as beta-lipoproteins and heavy immunoglobulins are present only in trace quantities. The albumin/globulin ratio of the aqueous is many times higher than that of plasma because of the exclusion of the heavy globulins from the aqueous by the blood-aqueous barrier. In patients with uveitis and aqueous humor-protein levels greater than 1 g/100 ml, there is total breakdown of the selectivity of the blood-aqueous barrier, and the aqueous humor protein fractions become qualitatively similar to those of plasma.[15] So, fibronectin in human aqueous humor may be produced by corneal endothelial cells or trabecular meshwork cells under normal conditions, and may be released from the ciliary body due to the breakdown of the blood-aqueous barrier under pathologic conditions such as uveitis.

The mechanism of higher concentration of fibronectin in glaucoma has not been clearly disclosed. It is thought that fibronectin may be accumulated because it can not pass the aqueous outflow pathway, or that fibronectin production may be increased in glaucoma.

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