A Study of Aqueous Humor Formation in Patients with Cystic Fibrosis

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The circadian pattern of aqueous formation and the effect of timolol on aqueous flow was studied in 12 patients with cystic fibrosis. Cystic fibrosis is a disease characterized by a defect in a chloride channel-associated regulatory protein found in epithelial cells. Improper regulation of these chloride channels, causes abnormal composition of exocrine secretions, including respiratory tract, gastrointestinal tract, exocrine pancreas, and sweat glands. Ocular findings previously reported include abnormal endothelial cell permeability, decreased tear secretion, and abnormal tear composition. In this study, aqueous humor flow was measured by fluorophotometry. No statistically significant difference was found when flow rates measured during the morning, during the afternoon, at night, and after topical timolol treatment were compared to normal values. The conclusion is that the beta adrenergically regulated chloride selective channels defective in patients in cystic fibrosis do not play a major role in the formation of aqueous humor or they are not regulated by the cystic fibrosis transmembrane conductance regulator (CFTR). Invest Ophthalmol Vis Sci 33:160–164, 1992

Cystic fibrosis is the most common serious inherited disease of whites, affecting 1 of every 2000 live births. It is inherited as an autosomal recessive trait. The defect is due to a mutation located in the middle of the long arm of chromosome 7. One out of 20 whites is a carrier. The disease is characterized by hyperviscous secretions leading to chronic pulmonary disease, pancreatic exocrine insufficiency,[1,2] and elevated levels of sweat chloride and sodium. It has been shown that other tissues, including salivary glands[3] and cervical,[4] nasal,[5] and tracheal epithelium,[6] also are affected. Thus, it is believed that the defect occurs in all types of epithelia.

In the eye, several abnormalities have been observed in patients with the disease. These include increased corneal endothelial permeability,[7] breakdown of the blood vitreous barrier in cystic fibrosis patients with diabetes,[8] decreased contrast sensitivity, preganglionic ocular sympathetic paresis,[9] decreased tear secretion,[10] and abnormal tear composition.[11]

The disease is characterized by a genetic defect in which the chloride selective channel of several types of epithelia cannot be gated by cyclic adenosine 3′,5′-monophosphate-dependent kinase.[12,13] The rate and the quality of secretion by the epithelium of the trachea are controlled by membrane chloride channels.[14] These channels open in response to stimulation of the cell by hormonal secretagogues, including beta-adrenergic agonists. In cystic fibrosis, the chloride channels are present in the cell membrane but they fail to respond to the kinase, suggesting a defect in the regulatory protein or a regulatory site of the channel protein itself. If stimulated by a large (unphysiologic) change in membrane potential, the channels exhibit the normal properties of conductance, ion selectivity, and voltage dependence. A recent finding links this defect to a deletion of a codon for the amino acid phenylalanine at position 508 (Δ_{F508}) of the cystic fibrosis transmembrane conductance regulator (CFTR). This defect is found in approximately 68% of defective genes of patients with cystic fibrosis.[15] In the remaining 32% of defective genes, an increasing number of mutations, now greater than 60, accounting for abnormal gene product are being identified. The Δ_{F508} deletion is postulated to prevent proper binding of adenosine triphosphate to a regulator protein of the chloride channel or to prevent the conformational change necessary for proper protein functioning, resulting in an insensitivity to activation by protein kinase A or C.[16,17]

It is plausible that this genetic defect can affect every secreting epithelial cell of the body, including secretory epithelia of the eye. This supposition has led us to design an experiment that would determine the role, if any, of beta-adrenergic regulation of chloride

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channels in the ciliary epithelium in producing or regulating the rate of formation of aqueous humor.

Aqueous humor is produced by the pars plicata of the ciliary body, a tissue that contains a double layer of epithelium. This tissue produces aqueous humor at a steady rate in human eyes during waking hours. During sleep, however, the rate of aqueous formation falls to approximately 50% of that observed during the day.[18] The diurnal increase in aqueous humor formation can be blocked by the topical administration of beta-adrenergic blocking agents, and the rate of aqueous humor formation during sleep can be stimulated by the topical application of several beta-adrenergic agonists.[19] These data, along with animal studies, collectively suggest that beta adreno-receptors are probably important in the regulation of aqueous humor formation.[20] If we hypothesize that the chloride channel whose regulatory mechanism is defective in cystic fibrosis is the principle ion channel responsible for the formation of aqueous humor, persons with cystic fibrosis might lack the normal circadian rhythm of aqueous humor flow and might be insensitive to beta-adrenergic antagonists. The study that follows was designed to test this hypothesis.

Material and Methods

Subject Selection

Fifty patients older than 14 years with a diagnosis of cystic fibrosis were identified from Mayo Clinic files, and additional patients were contacted through the Cystic Fibrosis Center of the University of Minnesota. A letter of invitation to participate in a two-day study was mailed to each of them.

Of eligible patients contacted, 12 (seven men and five women with an average age of 27) completed the study and are reported here. Each participant underwent a medical examination that confirmed the diagnosis of cystic fibrosis and ensured that the health was satisfactory for participation in the study. An ocular examination, including visual acuity testing, direct ophthalmoscopy, slit-lamp examination, and Goldmann's applanation tonometry, also was performed to rule out concurrent ocular disease. All federal guidelines for written informed consent were followed. The volume of the anterior chamber of each subject was measured photogrammetrically.[21] Each participant's blood was tested for the presence of the cystic fibrosis-associated mutation Δ_{F508} as described by Chong et al.[22]

The diagnosis of cystic fibrosis was confirmed if the participant had typical pulmonary manifestations, typical gastrointestinal manifestations, a history of cystic fibrosis in the immediate family, in addition to a sweat chloride or sodium concentration greater than 70 mEq.[2] Patients were considered too ill for study if they were dependent on supplemental oxygen or had signs of severe malnutrition.

Study Design

The study was performed during a two-day admission to the Clinical Research Center of the Mayo Clinic. Participants were admitted the evening before the first day of the study. Each subject was accommodated in a room located near the fluorophotometer, which was used for all measurements of aqueous humor flow. Each measurement requires approximately 5 minutes. Between measurements, the participants were unrestricted in activities, except they were instructed not to sleep during the waking portions of the study, to refrain from heavy exercise, and to avoid excessive eating or fluid intake. Meals and overnight accommodations were provided by the Clinical Research Center.

The baseline study consisted of measurement of the rate of aqueous flow at intervals over a 24-hour period, beginning at 8:00 a.m. The following morning, the effect of timolol on aqueous flow was measured between 8:00 a.m. and noon. Fluorescein was instilled on two occasions, first at 2:00 the morning of the baseline study day, and at 4:00 the afternoon of the same day. During the first day, the concentration of fluorescein in the cornea and anterior chamber was measured fluorophotometrically every hour from 8:00 a.m. to 4:00 p.m. At night, measurements were made at 10:00 p.m., 11:00 p.m., midnight, 6:00 a.m., and 7:00 a.m. The second day, measurements were made hourly from 8:00 a.m. until noon. Between midnight and 6:00 a.m. the subject was not disturbed. Subject were allowed to go back to sleep between 6:00 and 7:00 a.m., but after the 7:00 a.m. measurement they were asked to dress and eat breakfast. Immediately after the 8:00 a.m. measurement, timolol maleate 0.25% (Timoptic; Merck Sharp and Dohme, West Point, PA) was administered to one eye chosen by random; an identical-appearing placebo (Hypotears; IOLAB Corp., Claremont, CA) was administered to the fellow eye. Measurements continued hourly until noon. After the last measurement, tonometry was performed.

Calculation of Flow

The rate of flow was calculated as the rate of clearance of fluorescein from the combined cornea and anterior chamber on the assumption that 0.25 μl/min of the clearance was due to diffusional losses of fluorescein.[23] For the circadian pattern portion of the study, flow rates were calculated for the morning (8:00 a.m.–noon p.m.), the afternoon (noon–4:00 p.m.), for the entire day (8:00 a.m.–4:00 p.m.), and for the night

Table 1. Aqueous flow rates in patients with cystic fibrosis

	Flow (µl/min)					
	Circadian pattern				Timolol effect	
Genotype	Morning (8 AM–12 PM)	Afternoon (12 PM–4 PM)	All day (8 AM–4 PM)	Night (12 PM–6 AM)	Control (9 AM–12 PM)	Timolol (9 AM–12 PM)
$\Delta_{F508}/\Delta_{F508}$	1.70	2.19	1.94	1.234	2.06	1.53
$\Delta_{F508}/\Delta_{F508}$	3.52	3.08	3.30	1.03	2.53	1.77
$\Delta_{F508}/\Delta_{F508}$	2.75	2.68	2.71	0.75	1.33	0.73
$\Delta_{F508}/\Delta_{F508}$	2.21	2.02	2.12	1.49	2.00	2.20
$\Delta_{F508}/\Delta_{F508}$	3.34	2.58	2.96	1.25	2.25	2.00
$\Delta_{F508}/\Delta_{F508}$ Mean (SD)	2.71 (0.76)†	2.51 (0.42)†	2.61 (0.57)†	1.15 (0.28)†	2.03 (0.44)	1.64 (0.57)
Δ_{F508}/other	2.94	2.29	2.62	0.94	2.11	0.91
Δ_{F508}/other	2.15	2.31	2.23	1.24	2.78	1.58
Δ_{F508}/other	3.58	3.39	3.49	1.51	3.94	2.44
Δ_{F508}/other	2.76	2.61	2.68	1.18	2.02	1.53
Δ_{F508}/other	2.79	2.96	2.87	1.76	3.04	3.24
Δ_{F508}/other Mean (SD)	2.84 (0.51)†	2.71 (0.46)†	2.78 (0.46)†	1.33 (0.32)†	2.78 (0.78)	1.94 (0.91)
Other/other	2.80	2.51	2.65	1.61	2.04	1.54
Other/other	3.02	2.93	2.98	0.84	1.94	1.52
Other/other Mean (SD)	2.91 (0.16)†	2.72 (0.30)†	2.82 (0.23)†	1.22 (0.55)†	2.01 (0.04)	1.54 (0.02)
All CF pts Mean (SD)	2.80 (0.56)†	2.63 (0.40)†	2.71 (0.46)†	1.24 (0.31)†	2.27 (0.69)‡	1.69 (0.68)‡

* $P > 0.1$ compared to age-matched normals (Table 2).
† $P > 0.1$ compared to normal human volunteers (Table 2).

‡ $P = 0.0013$ comparing timolol-treated and untreated eyes.

(midnight–6:00 a.m.). For the timolol portion, flow rates were calculated for the period 9:00 a.m. to noon, allowing one hour for timolol to achieve its effect.

Statistical Analysis

Flow rates measured in the cystic fibrosis patients were compared to values of normal volunteers using a two-tailed t-test for independent samples. When comparing flow rates measured at different times of the day, and comparing timolol treated and untreated eyes, a one-tailed t-test for paired samples was employed.

Results

Table 1 is a summary of the rate of aqueous humor flow at different periods of the circadian cycle and in response to topical, unilateral timolol administration. The circadian pattern observed in patients with cystic fibrosis is normal. The flow is highest in the morning at 2.69 ± 0.67 µl min^{-1} (mean $\pm$ SD), not significantly changed at 2.55 ± 0.48 in the afternoon ($P = .19$), and lowest during sleep at 1.23 ± 0.30 ($P < 0.0001$). We compared these results to the flow observed in a large number of normal human subjects as well as in age-matched subjects from this larger group (Table 2).

Table 2. Aqueous flow rates in normal volunteers (flow µl/min)

	Circadian pattern			
	Morning (8 AM–12 PM)	Afternoon (12 PM–4 PM)	All day (8 AM–4 PM)	Night (12 PM–6 AM)
Age-matched with $\Delta_{F508}/\Delta_{F508}$ pts (n = 10)	3.19 (0.78)	2.69 (0.53)	2.94 (0.63)	1.14 (0.40)
Age-matched with Δ_{F508}/other pts (n = 10)	2.87 (0.40)	2.53 (0.43)	2.70 (0.39)	1.14 (0.44)
Age-matched with other/other pts (n = 4)	2.84 (0.16)	2.40 (0.14)	2.62 (0.15)	1.16 (0.25)
Age-matched with all CF pts (n = 24) Mean (SD)	3.00 (0.57)	2.57 (0.45)	2.78 (0.49)	1.14 (0.38)
Normal humans (day: n = 134, night: n = 67) Mean (SD)	2.86 (0.73)	2.63 (0.57)	2.75 (0.63)	1.18 (0.41)

These normal subjects were tested with the same procedure and equipment used in the present study. No differences were found in any of the time periods studied.

Table 3 is a summary of the response to the beta-adrenergic antagonist timolol. A 26% reduction in the treated eye compared to the fellow eye was observed as compared to a 30% reduction in a group of normal subjects studied by Topper et al.[24] The difference between the responses in the two groups is not statistically significant.

As summarized in Table 4, intraocular pressure (IOP) in cystic fibrosis patients does not differ significantly from normals, at baseline or in response to timolol. IOP at the screening eye examination was 13 ± 3 mmHg (mean ± SD) with a range of 9–18 mmHg. Four hours after topical instillation of timolol, IOP in the timolol- and placebo-treated eyes was 10 ± 3 mmHg and 14 ± 3 mmHg, respectively. This represents a 4 mmHg (29%) difference between treated and untreated eyes ($P < .0005$). The observed response is similar to the 3 mmHg difference (20%) found by Topper et al[24] using a similar study design.

Genetic analysis revealed that five of the subjects were homozygous for the Δ_{F508} mutation, five were compound heterozygotes, and two did not have the Δ_{F508} mutation on either allele. The compound heterozygotes and those without the Δ_{F508} mutation presumably carry one or two, respectively, of the other rarer mutations of CFTR gene.

Discussion

A minimum set of conditions required to cause a difference between patients with cystic fibrosis and normal subjects are as follows.

1. The beta adrenergically regulated chloride-selective channel defective in cystic fibrosis plays a major role in the formation of aqueous humor.
2. The response to timolol and/or the circadian rhythm in normals is mediated greatly via the ef-

Table 3. Response to Timolol in Cystic Fribosis and Comparison to Normals

	Aqueous humor flow (μl/min) mean ± SD	
	Timolol treated eye	Placebo treated eye
Cystic fibrosis (n = 12)	1.69 ± 0.68*	2.27 ± 0.69*
Normals (n = 19)†	1.58 ± 0.49	2.26 ± 0.86
Probability of Type I error	P > .05	P > .05

* $P = .0013$.
Probability of Type II error for finding 30% difference: $P < 0.01$.
† Topper and Brubaker, 1985.

Table 4. Intraocular pressure (mm Hg)

	Initial exam
CF patients	13 ± 3
Normals (n = 19)*	14 ± 3 ($P > 0.1$)
	After timolol
Control	14 ± 3
Timolol	10 ± 3 ($P < 0.005$)

* Topper and Brubaker, 1985.

fects of cAMP-dependent protein kinase on this chloride channel.
3. The mutant defect of cystic fibrosis, present in other epithelia, also is present in the ciliary epithelia.

Failure of any one of the three conditions listed above would be sufficient to account for the observation that aqueous humor flow, its circadian rhythm, and its response to timolol were found to be normal in these subjects. It seems unlikely, however, that ciliary epithelia would not be affected by the genetic defect, leaving some ambiguity between choices one and two. Thus, the experiment is inconclusive in determining the mechanism of timolol's effect or that of the circadian rhythm, but it appears to exclude the possibility that both conditions one and two are met.

Acknowledgments

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