Reduction of Collagen Type I in the Ciliary Muscle of Inflamed Monkey Eyes

Takeshi Sagara,1 Dan D. Gaton,1 James D. Lindsey,1 B’Ann True Gabelt,2 Paul L. Kaufman,2 and Robert N. Weinreb1

PURPOSE. To investigate in monkey ciliary muscle the relationship between the extent of anterior segment inflammation and alterations of collagen type I as determined by quantitative imaging densitometry.

METHODS. Anterior segment inflammation was induced in one eye of five cynomolgus monkey by cannulation of the anterior chamber, by anterior chamber injection of bovine serum albumin, or by disruption of the iris and anterior lens capsule with a needle. Increases in inflammatory cells were scored in hematoxylin and eosin-stained sections. Parallel eye sections were immunostained for collagen type I and developed using diaminobenzidine. Optical density (OD) was measured along two line segments overlaying the immunostained ciliary muscle using two-dimensional imaging densitometry. To assess antibody labeling of ciliary muscle structures, additional sections were double-immunostained using antibodies to collagen type I and calponin and examined by confocal microscopy.

RESULTS. In each of the inflamed eyes, hematoxylin and eosin-stained sections showed signs of chronic inflammation including lymphocytes and macrophages dispersed among ciliary muscle fibers and in the iris. Double label confocal microscopy showed collagen type I immunoreactivity in the interstitial extracellular matrix between bundles of ciliary smooth muscle fibers. Collagen type I OD scores in each of the inflamed eyes were less by 16% to 55%, compared with the contralateral control eyes. The mean of the OD scores for all inflamed eyes was 39% ± 7% less than the mean of the control eye scores (mean ± SEM, P < 0.001). Regression analysis showed a close correlation between inflammatory cell scores in the treated eyes and the reduction of OD scores (r = 0.94, P = 0.02).

CONCLUSIONS. These results indicate that the density of collagen type I in the extracellular matrix (ECM) of monkey ciliary muscle is reduced during anterior segment inflammation and support the view that reduction of ciliary muscle ECM may contribute to increased uveoscleral outflow facility during anterior segment inflammation. (Invest Ophthalmol Vis Sci. 1999;40:2568–2576)

Intracameral infusion of various tracers including thorotrast, gelatin, latex spheres, and ferritin has identified interstitial spaces between ciliary muscle bundles as the predominant route for uveoscleral outflow.1–5 These spaces contain various extracellular matrix molecules including collagen type I, collagen type III, and fibronectin.4,5 Toris and Pederson first reported increased uveoscleral outflow in monkey eyes with experimental autoimmune uveitis,6 a result that subsequently has been confirmed.7 In addition, histologic studies have revealed enlarged ciliary muscle interstitial spaces and suprachoroidal spaces in eyes with uveitis caused by intravireal bovine serum albumin (BSA) injection6 or by laser cyclophotocoagulation.8 These studies suggest that reduction of ciliary muscle extracellular matrix (ECM) may contribute to uveitis-linked increases of uveoscleral outflow by decreasing hydraulic resistance. However, the identity of ECM components within the uveoscleral pathway that are affected by iridocyclitis is not known.

The possibility that the reduction of collagen type I might be involved is supported by several lines of evidence. It has been known for more than 25 years that prostaglandin (PG) concentrations within aqueous humor are increased during inflammation.9 Administration of topical PGF2 isopropylester (PGF2,IE) increases uveoscleral outflow in cynomolgus monkey eyes.10,11 Anatomic studies have shown that ciliary muscle interstitial ECM is reduced in monkey eyes after repeated topical applications of PGF2,IE.4,12,13 and a major component of the interstitial ECM is collagen type I.5,14 Moreover, quantitative and qualitative investigations with specific antibodies have shown that collagen type I around cultured human ciliary muscle cells in vitro is both reduced and remodeled after the addition of certain prostaglandins.15 Thus, it is possible that ECM changes in the anterior segment with iridocyclitis may reflect changes in collagen type I within the ciliary muscle.

Quantitative immunocytochemical methods have been developed to measure the density of a number of different tissue...
antigens in vivo. One of the first methods used peroxidase-conjugated detection of bound antibodies and analyzed staining density with computer-assisted video densitometry. This and later studies have shown how increased signal-to-noise ratio could be obtained by optimizing immunoreagent concentration and incubation conditions. Recently, Huang et al. showed a significant positive linear relationship between optical density (OD) and antigen concentration within immunostained sections. In the present study, these methods were adapted for use with an imaging densitometer to assess the relationship between increased inflammatory cells and changes of collagen type I in the cynomolgus monkey ciliary muscle.

**METHODS**

**Experimental Design**

Paired eyes from 5 cynomolgus monkeys (Macaca fascicularis) were obtained from the University of Wisconsin–Madison. The monkeys were of both sexes and weighed 3.1 to 5.2 kg. All 5 monkeys had been previously used extensively for outflow facility experiments and other procedures, as described below. These animals had become largely unusable for aqueous dynamics experiments due to either inflammation in one eye or corneal cloudiness obstructing the assessment of anterior chamber (AC) clarity. Their assignment to the present study was consistent with good utilization and conservation of valuable primate resources.

The study objective was to evaluate monkey eyes in which biomicroscopic examination disclosed the presence of clinical signs of inflammation in one eye and no AC cells or flare in the other eye during 5 or more days immediately before the monkeys were killed. If signs of persistent inflammation were already present in one eye at the beginning of this study, no further manipulation was done. If biomicroscopic signs of inflammation were not present in either eye, one eye was treated to cause inflammation.

**Treatments and Biomicroscopic Assessment of Inflammation**

All 5 monkeys had been studied previously and had undergone between 6 and 18 prior AC perfusions with Bárány’s solution, in which various drugs were introduced into either eye and vehicle (Bárány’s solution + dimethyl sulfoxide) into the contralateral eye. Outflow facility was measured bilaterally. These drugs did not include prostaglandins or other agents known to alter collagen metabolism. In each monkey, the identity of the control and experimental eyes varied among the perfusion experiments performed. Monkey K392 also had undergone bilateral total iridectomy 8.5 months earlier and lens removal by endcapsular phacoemulsification 2.5 months earlier in the eye that was treated to induce inflammation, as described below. Each monkey was allowed a 4- to 6-week rest period between perfusion experiments to allow any inflammation resulting from AC cannulation and perfusion to subside. Before each of the above perfusion experiments, as well as before the present study treatments to induce inflammation, slit-lamp examination revealed all eyes to be free of AC cells and flare.

Monkeys K340 and K392 had inflammation induced in one eye by injection of 10 µl of 0.1% BSA dissolved in Bárány’s solution into the AC of one eye while the monkeys were under ketamine anesthesia (10 mg/kg, IM). Previous studies have reported that this treatment reliably produces inflammation in monkey eyes. Five days later, these animals were killed. Biomicroscopic examination of monkey K392 the day before they were killed showed +2 cells in the AC of the treated eye and a clear AC in the contralateral eye. The cornea of the treated eye of monkey K340 was edematous on the day before it was killed, which obscured viewing of cells in the AC. The contralateral eye was clear.

Inflammation was induced in monkeys K92 and K456 by inserting a needle into the AC, stroking the iris, and puncturing the lens 3 weeks before they were killed. In monkey K456, AC cells were observed in the treated eye 1 week after treatment. The AC was collapsed just before the animal was killed, suggesting that inflammation was present throughout the 3-week period. The contralateral eye was clear the day before it was killed. In monkey K92 only trace cells were detected 1 week after the first procedure, hence the treatment was repeated 1 week before the animal was killed. Biomicroscopic examination disclosed +1 cells in the AC, lens opacity with an iridolenticular adhesion in that eye, and a clear AC in the contralateral eye the day before the animal was killed.

Monkey K355 had developed iatrogenic inflammation in one eye after botulinum toxin injection into an extraocular muscle 1 month before the animal was killed. This inflammation was apparent clinically in excess of 2 weeks without further manipulation. One day before the animal was killed, biomicroscopic examination showed +1 cells in the AC, neovascularization in the cornea, and an iridolenticular adhesion. The AC of the contralateral eye was clear.

The magnitude of inflammation was characterized further in all eyes by scoring histologic sections of the ciliary muscle for increased inflammatory cells and for evidence of tissue reorganization. Adjacent sections were then analyzed for collagen type I changes in the ciliary muscle by quantitative immunohistochemistry.

**Tissue Processing**

The animals were killed by intravenous pentobarbital overdose. The eyes were enucleated and then hemisected through an equatorial plane. The anterior segments were immediately fixed in methacarn (60% methanol, 30% chloroform, 10% glacial acetic acid) for 3 hours. Increased sensitivity of immunohistochemical staining of various antibodies has been demonstrated for many antigens after methacarn fixation. The anterior segment tissue was then transferred to 100% ethanol and shipped by overnight express to University of California–San Diego. The tissues were embedded in paraffin, and sections were collected from the mid-sagittal region of each eye on Vectabond-coated slides (Vector Laboratories, Burlingame, CA). Initially, 4-µm sections were collected for a pilot study to determine which thickness yielded optimal immunostaining. As a result of this pilot study, subsequent sections for histopathologic and quantitative analyses were 12-µm thick. For histopathologic analysis, sections from each eye were stained with hematoxylin and eosin.

All procedures were conducted in accordance with the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.
Double-Label Immunohistochemistry

Deparaffinized anterior segments were double-immunostained using affinity-purified polyclonal rabbit anti-human collagen type I antibody (T61547R, diluted 1:50; Biodesign International, Kennebunk, ME) and monoclonal anti-human calponin antibody (clone hCP, diluted 1:1000; Sigma, St. Louis, MO). Specificity of each antibody has been previously confirmed. Calponin is a cytoplasmic actin-binding protein present in the ciliary muscle fibers. Secondary antibodies included Alexa 568-conjugated goat anti-rabbit immunoglobulin G (IgG) antibody (Molecular Probes, Eugene, OR, diluted at 1:200) and Alexa 488-conjugated goat anti-mouse IgG (diluted at 1:200; Molecular Probes). These sections were examined using a confocal laser scanning microscope equipped with an argon–krypton laser (FLUOVIEW; Olympus America, Melville, NY).

Quantitative Immunohistochemistry

Preliminary investigations of collagen type I immunoreactivity revealed that a substantial portion of the OD signal in the ciliary muscle was due to endogenous pigment. Moreover, in nonstained sections, it was apparent that adjacent histologic sections could contain varying amounts of pigment. Previous studies have shown that pigment can be extracted from eye sections with permanganate and oxalic acid. However, prolonged exposure to these agents can reduce the structural integrity of tissue sections. Hence, section thickness and solution parameters in these protocols were refined to maximize pigment extraction and reproducibility while retaining good tissue preservation.

Strict adherence to the following protocol was maintained. Five sections from each eye were heated to 56°C for 20 minutes, washed in three xylene changes to remove paraffin, and rehydrated through graded ethanol. The sections were treated with an antigen retrieval solution (AR-10; Biogenex, San Ramon, CA) at 95°C for 5 minutes. After cooling, the sections were blocked 30 minutes with 10% BSA (Sigma Chemical) and incubated with the polyclonal anti-rabbit antibody to human collagen type I (T61547R; Biodesign International) overnight at 4°C. After rinsing, the sections were blocked 30 minutes with 0.1% goes with antibody to collagen type I. The section had been treated to remove endogenous pigment and was not counterstained after immunostaining. Hence, all visible details in the section reflect collagen type I immunoreactivity. The stronger the staining intensity, the darker the image acquired from the densitometer. OD in the ciliary muscle was measured along two line segments positioned perpendicular to the ciliary body long axis as shown. Background noise measured along the third parallel line segment nearby, but not overlying the tissue, was defined as baseline OD and subtracted. Magnification, ×30. CM, ciliary muscle; IR, iris.

After immunostaining, the sections were scanned by laying the slides directly on the platen of a two-dimensional scanning densitometer (model GS-700 Imaging Densitometer; Bio-Rad, Hercules, CA). Because the sections were midsagittal, the ciliary muscle tissue examined was from the superior and inferior quadrants. Resolution of the scans was set to 1200 dpi (50-μm-wide pixels), and scanning mode was set to transillumination. The scanned digital data were displayed in a two-dimensional format and analyzed using an image analysis program (Molecular Analyst, version 2.1; Bio-Rad). The OD along two line segments positioned perpendicular to the long axis of the ciliary body and near the widest region of the ciliary muscle were measured in each section as shown in Figure 1. The positioning of these line segments over the ciliary muscle tissue avoided any remaining cluster of pigment granules. Background noise measured along one line segment nearby, but not overlying the tissue, was defined as baseline and subtracted from the final scores. The range of ciliary muscle width extended from the inner limit of sclera to the outer limit of muscle surface toward the ciliary pigmented epithelium. Ciliary pigmented epithelial cells, which showed a high peak on an image densitometry map, were excluded (Fig. 2). Likewise, the inner limit of sclera was detected readily in all sections by a step in OD. Specific OD score along each line segment over the ciliary muscle was calculated by dividing the OD area score (OD × mm, provided by the densitometer) by the length of the line segment (mm) for that score. The maximum width of the ciliary muscle in each section also was recorded. Mean specific OD scores from the inflated eye of each monkey was compared to its contralateral control eye using the paired Student’s t-test. The paired Student’s t-test was used to compare the mean of mean scores of all inflated and all control eyes. In each case, a P value ≤ 0.05 was considered significant. To assess the possibility that OD score changes might reflect swelling of the ciliary body, the widest portion of the ciliary
body in each eye was measured on the digitized image map. The mean of these lengths in the inflamed eyes was compared to that in the control eyes.

**Grading of Histopathologic Sections**

The extent of increased inflammatory cells was graded in hematoxylin and eosin–stained sections from all experimental eyes on a six-point scale as follows: 0, occasional nonclustered inflammatory cells including lymphocytes, macrophages, and polymorphonuclear lymphocytes; +1, nonclustered inflammatory cells accompanied by occasional clusters of inflammatory cells (<5 cells/cluster); +2, small clusters of inflammatory cells (maximal 5–10 cells/cluster); +3, small and large clusters of inflammatory cells (maximal >10 cells/cluster) but no change in tissue organization; +4, clusters of inflammatory cells (maximal >10 cells/cluster) with minor enlargement of the spaces between the muscle fibers; +5, clusters of inflammatory cells (maximal >10 cells/cluster) with major enlargement of spaces between the muscle fibers.

**RESULTS**

**Double Label Immunohistochemistry**

Confocal microscopy revealed multiform strips of collagen type I immunoreactivity within the interstitial ECM among bundles of ciliary smooth muscle fibers (Fig. 3). Uniform cytoplasmic calponin immunoreactivity was observed within smooth muscle fibers. Neither protein was detected within the pigment-containing cells adjacent to the smooth muscle fibers (Fig. 3D). These results are consistent with previous reports of collagen type I distribution in monkey ciliary muscle.\(^4\) The distributions of calponin and collagen type I were similar in the control and treated eyes.

**Histologic Assessment of Inflammation**

In each of the inflamed eyes, hematoxylin and eosin-stained sections showed increases in inflammatory cells including lymphocytes and macrophages dispersed among ciliary muscle fibers (Figs. 4B and 4D) and in stromal matrix adjacent to the head of the ciliary muscle (Fig. 4D). Isolated lymphocytes also were commonly seen adjacent to the ciliary pigment epithelium (Fig. 4F) and in the iris stroma (Fig. 4J). In eyes with increased inflammation, occasional clusters of lymphocytes were observed adjacent to ciliary pigment epithelium (Fig. 4I). Polymorphonuclear leukocytes were observed only within blood vessels (Fig. 4G). Signs of muscle atrophy, fibrosis, and hyalinization were not observed in these sections.
As shown in Table 1, the scores of the inflamed eyes ranged from +2 to +5. The scores for the monkeys that had received AC BSA injection both were +3. The scores for the monkeys that received needle stroking of the iris were +2 and +4. Occasional inflammatory cells were observed in the contralateral control eyes; however, there was no overlap between the lowest inflammed eye score and the highest control eye score.

**Quantitative Immunohistochemistry**

We found that basic antigen retrieval solution provided increased immunostaining compared with acidic or neutral antigen retrieval solutions. The antigen retrieval technique is a non–enzymatic antigen unmasking method used before immunostaining. To improve tissue preservation, we modified the high temperature microwave heating step that is recommended for formalin-fixed tissues, by reducing the solution temperature to 95°C. For good tissue preservation and good bleaching results, we found that 10 minutes in potassium permanganate (2.5 g/l) and 3 minutes in oxalic acid (5 g/l) were sufficient as suggested by Alexander et al. and similar to what Kaneoka et al. proposed. As shown in Figure 5A, bands of collagen type I immunoreactivity corresponding to the interstitial ECM were often seen in the ciliary muscle. Reduced collagen type I immunoreactivity was observed in the ciliary muscle from the inflamed eyes (Fig. 5B). In addition, characteristic collagen type I bundles were visualized in the sclera adjacent to the ciliary smooth muscle (Fig. 5D) and in the cornea (Fig. 5G). Generating solutions of 4 and 10 μm produced insignificant differences in the OD scores (Fig. 6). However, good tissue preservation was more consistently observed in the 10-μm-thick sections. Thus, 10-μm section thickness was chosen for subsequent analysis. Systematic reduction of primary antibody dilution produced saturating increases in the OD scores (Fig. 7). To maximize sensitivity, a submaximal staining dilution of 1:50 was chosen. The ciliary body width

![Image](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933584/ on 04/02/2017)

**TABLE 1. Histologic Grading of Inflammation in Experimental Eyes**

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Inflamed Eye</th>
<th>Control Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>K92</td>
<td>+4</td>
<td>0</td>
</tr>
<tr>
<td>K340</td>
<td>+3</td>
<td>+1</td>
</tr>
<tr>
<td>K355</td>
<td>+5</td>
<td>+1</td>
</tr>
<tr>
<td>K392</td>
<td>+3</td>
<td>+1</td>
</tr>
<tr>
<td>K456</td>
<td>+2</td>
<td>0</td>
</tr>
</tbody>
</table>

For key to grading see text.
inflamed eyes, 805 ± 23 μm, was not significantly different from corresponding measurements in the control eyes, 823 ± 11 μm (Table 2). This indicates that the scoring was minimally affected by ciliary body swelling.

Table 3 shows the collagen type I OD scores in each of the inflamed eyes, which were less by 16% to 55% compared with the contralateral control eyes. The mean of the OD scores for all inflamed eyes was 39% ± 7% less than the mean of the control eye scores (mean ± SEM, \( P < 0.001 \)). As shown in Figure 8, the magnitude of the collagen type I reduction was closely correlated with the magnitude of the inflammation score (\( r = 0.94, P = 0.02 \)).

**DISCUSSION**

In the present study, two-dimensional imaging densitometry was used to evaluate immunostaining intensity in the ciliary body of inflamed monkey eyes. Variation of OD scores due to intrinsic melanin was minimized by removal of the melanin before immunostaining. This approach facilitated both qualitative and quantitative analysis of end products of immunohistochemistry. Qualitative analysis provided the data on the location of immunopositive tissue components and allowed for accurate placement of the sampling cords for quantitative analysis of immunoreactivity. The appearance of collagen type I immunoreactivity only within the interstitial matrix of monkey ciliary muscle in the present study was similar to the distribution observed in a previous report as well in the interstitial matrix of human ciliary muscle, and is consistent with specific recognition of collagen type I in the present

**TABLE 2. Width of Ciliary Body in the Section**

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Control Eye</th>
<th>Inflamed Eye</th>
<th>( P ) via ( t )-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>K92</td>
<td>859 ± 34*</td>
<td>849 ± 18</td>
<td>0.79</td>
</tr>
<tr>
<td>K340</td>
<td>833 ± 13</td>
<td>835 ± 34</td>
<td>0.96</td>
</tr>
<tr>
<td>K355</td>
<td>800 ± 12</td>
<td>746 ± 13</td>
<td>0.02</td>
</tr>
<tr>
<td>K392</td>
<td>818 ± 20</td>
<td>844 ± 11</td>
<td>0.27</td>
</tr>
<tr>
<td>K456</td>
<td>806 ± 24</td>
<td>751 ± 16</td>
<td>0.07</td>
</tr>
<tr>
<td>Mean</td>
<td>823 ± 11</td>
<td>805 ± 23</td>
<td>0.50</td>
</tr>
</tbody>
</table>

* Mean ± SEM (in micrometers), \( n = 5 \).
study. This supports the appropriateness of assessing collagen type I content in the ciliary muscle based on the OD measurements. The absence of significant change of ciliary muscle width with inflammation indicates that the observed changes in collagen type I immunoreactivity did not reflect inflammation-associated tissue swelling. Also, the close correlation between the increases in inflammatory cells and the magnitude of collagen type I reduction supports a link between these parameters.

Reduction of ciliary muscle collagen type I with inflammation may contribute to changes in aqueous dynamics. Previous studies have noted that decreased intraocular pressure associated with anterior segment inflammation reflects both reduced aqueous humor formation and increased uveoscleral outflow. Because uveoscleral outflow passes through the ciliary muscle interstitial matrix, reduction of collagen within the ciliary muscle interstitial spaces could reduce the hydraulic resistance to uveoscleral outflow.

Inflammation has been associated with a reduction of collagen in a number of nonocular tissues. For example, reductions of collagen have been documented in inflamed dermal tissues, synovial and bone tissues, soft connective tissues, intimal and interstitial matrices of aortic aneurysms, and periodontal tissues. Reduction of collagens also has been observed in experimental models of inflammation in lung tissue, synovium, and dermal kerocytes. Thus, the present observations in inflamed ciliary muscle support a general role for reductions of collagen in association with inflammation. The generality of our results is further supported by the fact that the heterogenous methods used to induce ocular inflammation all resulted in a reduction in collagen.

The present observation that monkeys with unilateral ocular inflammation exhibited less collagen type I in the ciliary muscle of the inflamed eye than in the contralateral uninflamed eye suggests that reduction of collagen type I may underlie increased uveoscleral outflow. In support of this, we recently have found that twice daily topical treatment of cynomolgus monkey eyes with 2 µg PGF2α-isopropyl ester, a treatment that lowers intraocular pressure by increasing uveoscleral outflow, also reduced collagen type I immunoreactivity in the ciliary muscle. The method for quantitative analysis of immunoreactivity in this investigation was the same as in the present investigation. The mean magnitude of collagen type I immunoreactivity reduction, 52% ± 7% (SD), was associated with a mean intraocular pressure reduction of 9.0 ± 2.2 mm Hg (SD). Hence, the reduction of collagen type I that ranged up to 55% in the present study is likely to be associated with substantial intraocular pressure reduction due to increased uveoscleral outflow. In addition to reduction of collagen type I immunoreactivity, the present study also noted substantial reductions of collagen type III and collagen type IV in the PGF2α-isopropyl ester-treated eyes. Hence, many ECM components in the ciliary muscle may be altered in eyes with mild to moderate inflammation.

Although our study does not address the mechanism of the reduction of collagen type I, indirect evidence points toward induction of increased degradation by matrix metalloproteinases (MMPs) by inflammation-associated prostaglandins. Substantial evidence supports the release of prostaglandins into the aqueous humor during ocular inflammation. We have observed that treatment of human ciliary muscle cells in vitro induces increased release of MMP-1, MMP-2, MMP-3, and MMP-9. As in other inflamed tissues, MMPs released from inflammatory cells such as mononuclear macrophages also may contribute to ECM degradation. Supporting the role for MMPs in ocular inflammation, increased MMP-1, -3, and -9 has been reported in the aqueous humor from patients with uveitis and experimental animals with experimental autoimmune uveitis. Thus, an inflammation-associated reduction of tissue collagens may be caused by prostaglandin-mediated increases in MMP secretion by ciliary muscle fibers, as well as by infiltrating inflammatory cells.

### Table 3. Collagen Type I Optical Density Score Changes with Inflammation

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Control Eye</th>
<th>Inflamed Eye</th>
<th>% of Reduction</th>
<th>P via t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>K92</td>
<td>0.066 ± 0.001*</td>
<td>0.030 ± 0.002*</td>
<td>54 ± 3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>K340</td>
<td>0.022 ± 0.004</td>
<td>0.015 ± 0.007</td>
<td>32 ± 11</td>
<td>0.009</td>
</tr>
<tr>
<td>K355</td>
<td>0.029 ± 0.004</td>
<td>0.013 ± 0.003</td>
<td>55 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>K392</td>
<td>0.022 ± 0.004</td>
<td>0.014 ± 0.006</td>
<td>38 ± 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>K456</td>
<td>0.067 ± 0.003</td>
<td>0.057 ± 0.003</td>
<td>16 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean</td>
<td>0.041 ± 0.010</td>
<td>0.026 ± 0.008</td>
<td>39 ± 7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Mean ± SEM, n = 10.

Figure 8. Correlation between increased inflammatory cell score and the reduction of collagen type I in each monkey eye. The straight line was calculated using linear regression analysis.
Further study is required to determine the role of prostaglandins and MMPs in inflammation-mediated collagen remodeling in the ciliary muscle and to determine whether inflammation induces similar changes in other interstitial ECM components such as other collagen or fibronectin. Because MMP-2, -3, and -9 also can degrade components of basement membranes separating ciliary muscle interstitial ECM from the ciliary muscle fibers, it will be important to look for changes in basement membrane components such as laminin and collagen type IV. Moreover, changes in ECM components within other ocular tissues, such as the sclera, also may be important for uveoscleral outflow.

References


10. Nilsson SF, Samuelsson M, Bill A, Stjernschantz J. Increased uveoscleral muscle fibers, it will be important to look for changes in basement membrane components such as laminin and collagen type IV. Moreover, changes in ECM components within other ocular tissues, such as the sclera, also may be important for uveoscleral outflow.


