Relationship between Aqueous Humor Protein Level and Outflow Facility in Patients with Uveitis

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PURPOSE. To determine whether there is a relationship between the aqueous humor protein level and outflow facility in patients with uveitis.

METHODS. Aqueous humor protein levels were determined by laser flare photometry, and outflow facility was determined by Schiotz tonography.

RESULTS. Thirty patients with uveitis and 10 control subjects were studied. Outflow facility was lower in patients with uveitis (0.21 ± 0.12 μl/min · mm Hg) than in control subjects (0.33 ± 0.05 μl/min · mm Hg, P < 0.001). Patients with uveitis and laser flare photometry results (flare more than 20 photon units/msc (n = 21) had a lower outflow facility (0.17 ± 0.07 μl/min · mm Hg) than patients with uveitis and flare less than 20 photon units/msc (n = 9, 0.32 ± 0.14 μl/min · mm Hg, P = 0.004). Furthermore, no difference was identified between outflow facility in patients with active uveitis (those who had anterior chamber cells) and flare less than 20 photon units/msc and outflow in control subjects. In patients with uveitis, there was a linear correlation between flare and outflow facility (r = −0.50, P = 0.005). There was no relationship between flare measurements and either intraocular pressure or aqueous humor cell levels when scored with a clinical, semiquantitative system.

CONCLUSIONS. Outflow facility is significantly reduced in patients with uveitis who have high aqueous humor protein levels. Outflow facility appears to be normal in patients with active uveitis whose flare levels are low, and therefore the association between flare and outflow facility does not appear to be an indirect reflection of elevated anterior chamber cells. It is possible that elevated aqueous humor protein levels contribute to the development of uveitic glaucoma in some individuals by decreasing aqueous humor outflow facility, although a causal relationship cannot be established on the basis of this study. (Invest Ophthalmol Vis Sci. 2001;42:2584–2588)
comparisons. Photometry readings are expressed as photon units per millisecond. Readings cannot be translated directly into aqueous humor protein concentration without knowledge of the protein concentration.7

Schiotz tonography was performed with the patient supine, according to previously described methods,8 without the investigator’s having knowledge of the flare measurements. The initial Schiotz scale reading and final scale reading were recorded. Measures of outflow facility were then obtained from previously published tables.8

Statistical Analysis

Comparisons between groups were made with Wilcoxon rank-sum test and the Fisher exact χ² test. A linear regression analysis was used to examine the relationship between outflow facility and laser flare photometry readings (flare). \( P < 0.05 \) was considered to be statistically significant.

RESULTS

There were 30 patients with uveitis and 10 control subjects enrolled in the study. All patients had evidence of active anterior segment inflammation, as evidenced by the presence of cells in the anterior chamber on slit lamp biomicroscopic examination (range, occasional to 3+). The anatomic categories of uveitis were as follows: anterior, 16 patients; intermediate, 2; anterior and intermediate, 1; panuveitis, 10; and posterior uveitis with an anterior chamber “spillover” reaction, 1. International Uveitis Study Group categories refer to the site of primary inflammation and, for the posterior and intermediate categories, do not preclude the additional presence of anterior segment inflammation.

Table 1 lists the demographic and medical characteristics of subjects. There were no significant differences in age or intraocular pressure between groups. There was a weak relationship between gender and, with a higher proportion of women in the group of patients with uveitis \( (P = 0.066) \). Although groups were not well matched for gender, we found no evidence in subgroup analyses that gender influenced levels of intraocular pressure, aqueous humor outflow facility, or flare in either the uveitis group \( (all \ P > 0.11) \) or the control group \( (all \ P > 0.44) \).

Among patients with uveitis, five subjects had uveitis of less than 3 months’ duration, whereas 25 subjects had uveitis of more than 3 months’ duration. Comparison of these two groups revealed no differences in intraocular pressure \( (P = 0.34) \), outflow facility \( (P = 0.44) \), or laser flare photometry readings \( (P = 0.87) \).

Outflow facility was significantly lower in patients with uveitis \( (0.21 ± 0.12 \mu l/min \cdot mm Hg) \) than in control subjects \( (0.33 ± 0.05 \mu l/min \cdot mm Hg, P < 0.001) \). The mean outflow facility observed in our control group was similar to that reported in previous studies for individuals without ocular disease.9,10 There was a wide range of laser flare photometry readings in the patients with uveitis \( (range, 4.6–193 \mu l/min\cdot mm Hg) \). All control patients had mean laser flare photometry results of less than 10 photon units/msec.

In the patients with uveitis, there was a statistically significant inverse relationship between outflow facility and laser flare photometry readings \( (r = −0.50, P = 0.005) \). The linear progression model shows that outflow facility = \(-0.0009 \times \text{flare} + 0.2814 \) \( (R^2 = 0.2489; \text{Fig. 1}) \). No relationship was identified between the semiquantitative score for anterior chamber cells and outflow facility.

Six patients with uveitis were being treated with topical aqueous humor suppressants (topical timolol or oral acetazolamide). All six had laser flare photometry readings of more than 20 photon units/msec. Because aqueous humor suppressants have been shown to increase flare readings,11,12 we evaluated patients with uveitis for an effect of timolol or acetazolamide on these readings, and to determine whether use of these drugs influenced the relationship identified between laser flare photometry results and outflow facility. Patients with uveitis who were receiving these drugs had slightly higher laser flare photometry readings \( (98.3 ± 57.5 \mu l/min\cdot mm Hsec) \) than those who were not \( (72.6 ± 65.5 \mu l/min\cdot mm Hsec) \), but the difference was not statistically significant \( (P = 0.256) \). After adjustment was made for the use of these drugs in the linear regression model, flare readings continued to have a significant effect on the slope estimate \( (P = 0.008) \), whereas use of aqueous humor suppressants did not \( (P = 0.351) \).

Topical corticosteroid medications were being used to treat 27 of 30 patients with uveitis at the time of the measurements. There was no linear correlation between the daily frequency of topical corticosteroid instillation and outflow facility \( (R = −0.13, P = 0.481) \). Of the three patients who were not treated with corticosteroids, two had laser flare photometry measurements less than 20 photon units/msec. There was a positive linear correlation between the daily frequency of topical corticosteroid instillation and flare \( (R = 0.39, P = 0.055) \), attributable to the fact that treatment is based, in part, on the severity of inflammatory signs. Nevertheless, when the daily frequency of topical corticosteroid instillation was adjusted in the linear regression model, the effect of flare was almost the same as seen in the unadjusted model \( (slope = −0.00096, P = 0.007) \), whereas no effect of daily frequency of topical corticosteroid instillation was seen \( (slope = 0.001, P = 0.701, R^2 = 0.2530) \). Thus, daily topical corticosteroid instillation did not change the relationship between laser flare photometry results and outflow facility.

As noted in Figure 1, patients tended to cluster at the ends of the range of laser flare photometry readings, with a substantial number of patients having results of less than 20 photon
units/msec. It has been our clinical experience that readings below this level are usually not associated with appreciable clinical flare on slit lamp biomicroscopy and that patients with low readings have low rates of other uveitis-associated complications. We therefore compared aqueous humor outflow facility between patients with uveitis and low laser flare photometry readings (<20 photon units/msec, n = 9) and those with high readings (>20 photon units/msec, n = 21). Patients with low readings had significantly higher outflow facility (Table 2).

We identified no difference in outflow facility between patients with uveitis and low laser flare photometry readings (0.32 ± 0.14 μl/min/mm Hg) and control subjects (0.33 ± 0.05 μl/min/mm Hg, P = 0.561). Although we had low power to confirm that the small observed difference was real, we had a power of 80% to confirm that outflow facility in the group of patients with uveitis and low laser flare photometry readings was not reduced by more than 24% from the mean value of the control group. Furthermore, we had a power of 99% to confirm that the outflow facility in this group was not reduced more than 36% from the mean reading in the control group, meaning that it was higher than the mean reading observed in all patients with uveitis in this study.

All control subjects had laser flare photometry readings less than 10 photon units/msec. It is unusual for flare to be below this level in patients with active uveitis. Only three patients with uveitis were identified in this study who had readings of approximately 10 photon units/msec or lower. Two of the three patients were being treated with topical corticosteroids, and each of the three had outflow facility above the mean for the normal control subjects.

### DISCUSSION

It is assumed that alterations in aqueous humor outflow account for increased intraocular pressure in the majority of patients with uveitis in whom secondary glaucoma develops. Intraocular pressure can be defined by the equation $IOP = F/C + P_v$, where $F$ is aqueous production, $C$ is outflow facility, and $P_v$ is episcleral venous pressure. Although aqueous hypersecretion would result in elevated intraocular pressure if outflow facility were to remain unchanged, inflammation of the ciliary body typically results in decreased aqueous production, a fact that has been demonstrated in experimental models of uveitis. When aqueous hypersecretion is observed in forms of experimental uveitis, it is a transient phenomenon that gives way to hyposecretion. Also, the existence of aqueous hypersecretion as a mechanism for secondary glaucoma in clinical situations has been questioned.

There are a variety of potential mechanisms by which outflow facility might be reduced in patients with uveitis. Mechanical obstruction of the anterior chamber angle can result from pupillary block with iris bombe, peripheral anterior synechiae, or forward rotation of the ciliary body. Other proposed mechanisms include blockage of or damage to the trabecular mesh-

| Table 2. Comparison in Patients with Uveitis of Low Versus High Laser Flare Photometry Readings |
|-----------------------------------------------|------------------|------------------|------------------|------------------|
| Age (y)                                      | 41.6 ± 17.9‡     | 39.1 ± 11.1‡     | 0.903§           |
| Clinical cells (median)                      | 1+ (occasional-2+) | 1+ (occasional-3+) | 0.777¶          |
| Clinical flare (median)                      | 1+ (0-2+)        | 2+ (trace-3+)    | 0.310∥          |
| Laser flare photometry reading               | 12.0 ± 4.3       | 105.9 ± 56.0     | 0.867§           |
| Intraocular pressure (mm Hg)                 | 14.3 ± 2.6       | 14.9 ± 6.2       | 0.004¶           |
| Outflow facility (μl/min · mm Hg)            | 0.32 ± 0.14      | 0.17 ± 0.07      | 0.004¶           |

* Defined as laser flare photometry reading of <20 photon units/msec.
† Defined as laser flare photometry reading of >20 photon units/msec.
‡ Mean ± SD.
§ Wilcoxon rank-sum test.
¶ Fisher exact χ².
work by cells or protein.\textsuperscript{3,4} Whereas pupillary block and peripherally anterior synechiae can be excluded by careful examination, obstruction of the trabecular meshwork by cells and small molecules would be difficult to identify clinically.

Evidence from animal studies has demonstrated that polymorphonuclear leukocytes can infiltrate the trabecular meshwork and possibly lead to obstruction.\textsuperscript{5} The average pore size of the trabecular meshwork is 2 $\mu$m\textsuperscript{6} and a leukocyte is approximately 7 to 9 $\mu$m.\textsuperscript{7} Thus, although leukocytes can become deformed, an obstructive phenomenon may occur with a cellular reaction in the anterior chamber. We found no relationship, however, between outflow facility and cells, when graded with a semiquantitative system. We cannot rule out the possibility that a relationship between cells and outflow facility could be identified with more precise measurements, but a practical technique for such precise measurements is not available. Laser photometry cell counts are associated with wide SDs when specific cells are counted\textsuperscript{8} and are not considered to be reliable for these types of precise comparisons. Nevertheless, our study suggests that the role of cells in changing outflow facility may be small or nonexistent, because patients with uveitis and low flare had normal outflow facility, despite the presence of cells on clinical examination. It is possible, however, that cells play a role only if they adhere to and disrupt the trabecular meshwork. Case reports have described patients with evidence of cellular precipitates on the trabecular meshwork with elevated intraocular pressure, despite minimal anterior chamber cellular reaction.\textsuperscript{9} Thus, the amount suspended within the aqueous humor may not be relevant. The precipitates disappeared and the pressure decreased after treatment with topical corticosteroids. The effect on outflow facility was not investigated in these studies, however.

It has also been proposed that trabeculitis, or inflammation of the tissues that form the trabecular meshwork, can lead to trabecular dysfunction and obstruction to aqueous humor outflow in some patients with uveitis. Hogan et al.\textsuperscript{10} described an enucleated eye from one patient with herpetic uveitis and glaucoma in which there were thick edematous bands of tissue and inflammatory cells within the trabecular meshwork. Animal studies of herpetic uveitis have demonstrated inflammatory cells in the trabecular meshwork.\textsuperscript{11}

We chose to study elevated aqueous humor protein as an alternative explanation for reduced aqueous humor outflow. Laser flare photometry is a noninvasive technique that can be used to quantify the amount of protein within the aqueous humor.\textsuperscript{12} Multiple studies have demonstrated that protein concentration is linear in relation to laser flare photometry readings, both in vitro and in vivo.\textsuperscript{13} Thus, this technique allowed us to make precise comparisons of aqueous humor protein levels with measurements of outflow facility in patients with uveitis. Patients enrolled in this study demonstrated a wide range of laser flare photometry readings, consistent with ranges reported for other heterogeneous groups of patients with uveitis.\textsuperscript{14,15} Our results demonstrate that there is a relationship between aqueous humor protein concentration and outflow facility in patients with uveitis. Furthermore, laser flare photometry readings do not seem to be simply indirect markers of alterations in aqueous humor outflow by other factors related to uveitis, because outflow facility in patients with active uveitis and low laser flare photometry readings was similar to that of control subjects, even though these patients had as many as 2 $+$ cells detected in clinical examination. This observation adds strength to the hypothesis that an increased protein level may play a causal role in lower outflow facility.

Although we had insufficient statistical power to identify whether there were small differences in outflow between normal control subjects and patients with uveitis and low flare, we had sufficient statistical power to state that outflow facility was not reduced by more than 24% of normal levels. In previous studies of outflow facility in patients with open-angle glaucoma, these levels were approximately 40% lower than in age-matched control subjects.\textsuperscript{16}

Epstein et al.\textsuperscript{17} support the hypothesis that elevated aqueous humor protein may lead to changes in outflow facility. They perfused enucleated eyes with various dilutions of serum and found decreases in outflow facility. Serum diluted to 1.3 g protein/100 ml resulted in a 36% decrease in aqueous outflow facility. This protein concentration is within the range observed in patients with uveitis.\textsuperscript{18,19} They also noted that the obstruction of flow could not be altered by a saline washout and was not proportional to the viscosity of the perfusate, which led them to believe that the protein was either deposited on or adsorbed into the trabecular meshwork. It has been suggested that the scavenging ability of the endothelial cells may become overwhelmed by various materials, which leads to disorganization of the trabecular meshwork and obstruction of outflow.\textsuperscript{20,21} Support for this hypothesis comes from histologic samples from patients with pigmentary glaucoma, in which pigment-laden endothelial cells did not maintain normal trabecular architecture.\textsuperscript{22} Perhaps high levels of protein result in similar changes.

Laser flare photometry does not identify the nature of the protein changes in aqueous humor. It is believed that an increasing proportion of higher molecular weight proteins increases flare.\textsuperscript{23} Thus, the higher flare readings may indicate the presence of larger molecular weight proteins that may have a greater effect on outflow. The relationship between outflow facility and protein may be more complex than a simple bulk protein effect, however. McEwen\textsuperscript{24} has argued that the presence of proteins in aqueous humor has no effect on its viscosity as it passes through the trabecular meshwork. As an alternative mechanism, increased protein levels may include an outpouring of regulatory proteins that have an effect on the cytoskeletal regulations of aqueous humor outflow through the trabecular meshwork.\textsuperscript{25}

Fautsch et al.\textsuperscript{26} have shown that the product of the gene MYOC, which can be found in the aqueous humor, increases outflow resistance when infused into anterior segments, using a perfusion organ-culture technique in human eyes. Other proteins, such as recombinant $\beta$-galactosidase, bovine serum albumin, and fetal calf serum did not increase outflow resistance in the same system. Also, heat-denatured MYOC protein did not increase outflow resistance, suggesting that outflow resistance is related to an active property of the protein. It is likely that these proteins are not of sufficient amount in clinical disease to alter photometry measurements themselves, however. With regard to the MYOC protein, we found no references on a computerized literature search (Medline, provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD, and available at http://www.ncbi.nlm.nih.gov/PubMed/) for its concentration in aqueous humor of patients with uveitis. Various regulatory cytokines, however, are found in concentrations ranging from 5 to 500 $\times$ $10^{-6}$ $\mu$g/100 ml within the aqueous humor of patients with uveitis.\textsuperscript{27,28} In contrast, the total aqueous humor protein concentration in normal eyes ranges from 10 to 20 mg/100 ml,\textsuperscript{29} and in patients with uveitis, it can increase to levels as high as 2300 mg/100 ml.\textsuperscript{30} In one study, mean total aqueous humor protein in a group of patients with chronic uveitis ranged from 62 to 388 mg/100 ml (mean, $>166.8 \pm 132.8$ mg/100 ml). Albumin and immunoglobulins accounted for more than 90% of the proteins present.\textsuperscript{31} We also found, by regression analysis, no evidence that topical corticosteroids had a direct effect on outflow facility.

The spread of measurement values around the regression line in Figure 1 may simply reflect the imprecision of photometry or Schiotz tonography measurements or both. Alterna-
tively, it may be that change in outflow is a dynamic process occurring over time, as might be seen with protein regulation of trabecular function. Thus, outflow facility may not always reflect the aqueous humor protein levels at the same time point. In support of this concept, we observed one of our patients with HLA-B27-associated anterior uveitis serially and noted that improvement in outflow facility lagged behind the declining aqueous humor protein levels over several visits. We did not, however, routinely measure changes in laser flare photometry results over time in patients with active disease, and thus we do not know precisely the temporal relationship between changes in aqueous humor protein levels and decreased outflow facility or the duration of outflow changes. Nevertheless, a relationship between laser flare photometry results and outflow appears to be present both in patients with recent onset of uveitis and in those with chronic disease. Our groups were not matched for gender, but we found no evidence that gender affected outflow facility. Previous studies have shown no effect of gender on laser flare photometry results.24

There was no correlation between outflow facility and intraocular pressure, which may be explained by use of glaucoma medications, or, in the absence of such drugs, by alterations in aqueous humor production or other compensatory mechanisms, such as increased uveoscleral outflow, that may occur in patients with uveitis. None of the patients in this study had elevated intraocular pressure or glaucoma, although six patients were being treated with aqueous humor suppressants.

Whether changes in aqueous humor outflow facility of the magnitude seen in this study play a role in the pathogenesis of uveitic glaucoma remains unknown. The regulation of intraocular pressure is multifactorial, and the interaction of factors in patients with uveitic glaucoma is undoubtedly complex, but this study suggests that increased aqueous humor protein levels may play a role in some cases through its effect on outflow facility. Aqueous humor protein should be included among the factors investigated in future studies of uveitis and glaucoma.

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References