Effects of Acetazolamide on Passive and Active Transport of Fluorescein across the Normal BRB

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PURPOSE. To investigate the effect of the carbonic anhydrase inhibitor acetazolamide (AZM) on passive permeability and active transport of fluorescein across the blood-retina barrier in healthy subjects. The study may have implications for the understanding of the edema-reducing effect of AZM.

METHODS. The effect of AZM on the blood-retina barrier function was assessed by differential vitreous spectrofluorometry using fluorescein as a tracer. The study included fourteen healthy subjects in a randomized double-masked crossover trial with 3 days' treatment with AZM (500 mg/d) and placebo, respectively. The two examinations were separated by at least 1 week. Fluorescein concentration was determined separately from its metabolite fluorescein glucuronide. The passive permeability of fluorescein was determined by computerized modeling and curve-fitting to the preretinal curve and the plasma concentration curve obtained at 30 to 60 minutes after the injection of fluorescein. The unidirectional permeability due to outward active transport from vitreous to blood was estimated from the preretinal gradient and the plasma concentration at 7 to 10 hours after injection.

RESULTS. Treatment with AZM was associated with significant increases in passive permeability and unidirectional permeability of fluorescein. For the passive permeability the increase was on average $0.3 \pm 0.4$ nm/s (mean $\pm$ SD; range, $-0.8$ to $1.0$ nm/s), and for the unidirectional permeability the increase was on average $7.4 \pm 7.0$ nm/s (mean $\pm$ SD; range, $-3.3$ to $19.0$ nm/s).

CONCLUSIONS. Acetazolamide caused an increase in passive permeability. Unidirectional permeability was increased by AZM, indicating a stimulation of the outward active transport of fluorescein. It has been proposed that the edema-reducing effect of AZM is due to stimulated ion and fluid removal from the retina to the choroid. The results of this study are consistent with AZM affecting the blood-retina barrier with stimulation of at least one ion transport mechanism. (Invest Ophthalmol Vis Sci. 1999;40:1770–1775)

The carbonic anhydrase inhibitor acetazolamide (AZM) reduces macular edema as assessed by fluorescein angiographic leakage and improves visual acuity in some patients with macular edema related to certain inflammatory and degenerative eye diseases, including chronic iridocyclitis and retinitis pigmentosa complicated by macular edema. In retinitis pigmentosa, the carbonic anhydrase inhibitors AZM and methazolamide are the only drugs that have a documented beneficial effect on visual acuity. The mechanism is incompletely understood.

Vitreous fluorometry has documented that AZM reduces blood-retina barrier leakage in uveitis and in retinitis pigmentosa complicated by macular edema. Vitreous fluorometry has also been used to estimate the elimination kinetic of fluorescein from vitreous to blood. Differential spectrofluorometry enables determination of fluorescein and its metabolite fluorescein glucuronide in the vitreous and in the blood. Engler et al. used this modified vitreous fluorometry technique to obtain a more precise assessment of the carrier-mediated outward transport of fluorescein. We have recently examined the effects of AZM in nine patients with retinitis pigmentosa and macular edema using this method. Results from that study indicated that AZM stimulates active transport of fluorescein from retina to blood. To improve our understanding of the mechanism whereby AZM reduces retinal edema, the effects of AZM on passive permeability and active transport of fluorescein in healthy subjects were investigated in this study.

METHODS

Study Design

The effects of AZM on the passive permeability and unidirectional permeability due to outward active transport of fluorescein across the blood-retina barrier were assessed in a randomized double-masked placebo-controlled crossover study. The healthy subjects were randomized by drawing numbered packages with prepacked medicine for the first and second treatment periods, half of which had placebo for the first period, and half of which had AZM as the first drug. The coding was performed by a person who did not participate in the study in any other capacity.
The first permeability investigation occurred on day 4, after the intake of one capsule containing AZM (Diamox Retard, 250 mg) or placebo in the morning, in the evening the first 3 days, and in the morning the fourth day. After an interval of 3 to 14 days with no treatment, the second period of treatment commenced, with the second and final examination on day 4. Thus, the washout period between each permeability investigation was at least 1 week.

When the passive permeability and unidirectional permeability of fluorescein had been calculated from the data, the code was broken, and the AZM concentrations in frozen plasma samples were measured (analysis conducted at the National Poisons unit, Guy's & Street Thomas' Hospital, London, UK) to ensure that the drug had been taken as prescribed.

The research followed the tenets of the Declaration of Helsinki, and the protocol was approved by the Local Ethics Committee and the National Board of Health.

**Determination of Passive and Unidirectional Permeability**

Fluorescein is assumed to be transported from the blood to the retina by pure passive diffusion as previously discussed. Based on animal studies, the transport of fluorescein in the direction from retina to blood is assumed to include active transport. To our knowledge there is no evidence of other contributors to the transport of fluorescein across the blood-retina barrier. A significant posteriorly directed bulk flow has been refuted in several studies. Any contribution to the transport of fluorescein due to an electrical potential is supposed to be small.

The method and assumptions for determination of the passive permeability due to passive diffusion have previously been described in detail. The method used to quantify the unidirectional permeability due to active transport is with some modification identical to the method developed by Engler et al. A summary follows below.

**Instrumentation**

Ocular fluorescence measurements were performed by use of a differential spectrofluorometry technique using a commercially available fluorometer (Fluorotron, Ocumenics, CA) equipped with a modified light source that excites light at rapidly interchanging wavelengths of 458 and 488 nm. For the plasma fluorescence analysis a cuvette spectrophotometer (Perkin-Elmer LS 50) was used.

**Procedure**

All investigations commenced in the morning. An intravenous (IV) catheter was positioned in a superficial antecubital arm vein, and the pupils were dilated using topical 10% phenylephrine plus 1% tropicamide. Ocular fluorescence measurements were conducted before the injection of an IV bolus injection of sodium fluorescein (14 mg/kg body weight) and 30 minutes and 1, 7, 8, 9, and 10 hours after injection (three scans per measurement until 2 hours after injection, thereafter 4 scans per measurement). Sufficient dilation of the pupil (more than 7 mm) was ensured before every fluorescence measurement by supplementary eye drops. The fluorescein concentration in plasma was measured from blood samples drawn from the catheter before injection, after approximately 5, 7, 10, 15, and 30 minutes, and after 1, 2, 7, and 9 hours after injection. From each blood sample the first 10 ml was thrown out. For the blood samples, heparin-coated vials were used. The blood samples were centrifuged for 15 minutes at 3000 rpm (100g) followed by ultrafiltration of the plasma for 20 minutes by centrifugation at 5000 rpm (100g) with ultrafiltration filters, nominal cutoff at 30,000 Da (Millipore). The ultrafiltrate was frozen immediately for later analysis. A plasma sample taken before the injection of fluorescein was frozen to determine the AZM concentration later.

**Plasma Concentration Determinations**

Diluted samples of ultrafiltrate were examined by use of a cuvette spectrophotometer with excitation at 458 and 488 nm and emission at 515 nm. The concentration of fluorescein in plasma ultrafiltrate was determined separately from that of fluorescein glucuronide by use of differential spectrofluorometry.

**Vitreous Concentration Determination**

Data analysis included alignment of the scans after fluorescence landmarks (cornea and the lens), correction for autofluorescence of the eye, correction for absorption in the lens based on calculations of the lens transmittance, and correction for absorption in extrinsic fluorophores. The lens transmittance was calculated from an average of lens autofluorescence data from the first and second investigations and assuming an intrinsic ratio between the front and back lens peaks of 1.2. Ocular fluorescence data were used to calculate fluorescein concentration separately from fluorescein glucuronide as described by Larsen et al. Fluorescence coefficients for fluorescein and fluorescein glucuronide were determined on the basis of measurements of fluorescence in cuvettes with known concentrations and physiological pH (pH, 7.34).

**Mathematical Analysis of Passive Permeability**

Passive permeability, $P_{\text{pass}}$, across the blood-retina barrier and the vitreous diffusion coefficient, $D$, was calculated as average values based on the plasma concentration decay curves and the ocular axial fluorescence scans recorded at 30 minutes and 1 hour after fluorescein injection (totally 6 scans) by applying a numerical solution to a mathematical diffusion model. The calculations are based on the assumption that the barrier between blood and vitreous consists of a homogeneous spherical shell of negligible thickness and that the concentration profiles for fluorescein within the first hour are determined by the passive permeability, the concentration curve for non–protein bound plasma fluorescein, and the diffusion characteristics of fluorescein in the vitreous. The unidirectional flux due to outward active transport of fluorescein is assumed to be negligible within the first hours.

**Mathematical Analysis of Active Transport**

The unidirectional permeability of fluorescein in the direction from the retina to the blood, $P_{\text{mono}}$, was determined from scans recorded at 7, 8, 9, and 10 hours after fluorescein injection (4 scans per hour per eye), where the fluorescein concentration decreases toward the retina and the net transport of fluorescein is from the vitreous to the blood. The calculations assume that $P_{\text{mono}}$ is independent of the vitreous concentrations at the late measurements. Nonsaturated active transport has been assumed in other studies within this area.
The flux of fluorescein across the blood-retina barrier in the direction from the vitreous to the blood at the time, \( t \), is the sum of passive flux, \( J^{\text{pas}}(a) \), and the unidirectional flux,\(^1\) \( J^{\text{uni}}(a) \). The outward flux across the barrier was determined based on Fick’s law of diffusion from the vitreous concentration gradient at the retina surface and the diffusion coefficient of fluorescein in the vitreous.\(^2\) In the spherical eye with radius \( 0 \leq r \leq a \), where \( \partial C/\partial r \) denotes the preretalinal gradient in the distance, \( a \), the outward flux at the time \( t \) can be expressed as follows:

\[
-D(\partial C/\partial r)_a = J^{\text{pas}}(a, t) + J^{\text{uni}}(a, t)
\]

The passive and unidirectional fluxes can be expressed as

\[
J^{\text{pas}}(a, t) = P^{\text{pas}}\left(C(a, t) - C'(t)\right)
\]

\[
J^{\text{uni}}(a, t) = P^{\text{uni}}C(a, t)
\]

where \( C(a, t) \) and \( C'(t) \) are the concentrations at the surface of the retina and the fluorescein plasma concentration at the time \( t \), respectively. \( P^{\text{uni}} \) can be derived from these equations:

\[
P^{\text{uni}} = -D(\partial C/\partial r)C(a, t) + P^{\text{pas}}C'(t)/C(a, t) - P^{\text{pas}}
\]

As illustrated in Figure 1 the preregional gradient (in the direction from the vitreous to the retina) was estimated by linear regression to vitreous concentration data at the distance of 1 to 5 mm from the retina, and the concentration at the surface of the retina was calculated by linear extrapolation of the regression line. The plasma concentrations were measured at 7 and 9 hours. The concentration at 8 and 10 hours was determined by linear inter- and extrapolation, respectively. The average value of the diffusion coefficients for the two periods was used in the calculations. \( P^{\text{uni}} \) was calculated as an average of the values determined at 7, 8, 9, and 10 hours.

### Statistical Analysis

Statistical analysis was performed on the average values of the right and left eyes. Two sample tests (here the Wilcoxon rank sum test with continuity correction) between the two groups defined by the treatment order were performed, comparing the period differences within each patient (test for treatment effect), treatment differences (period effect), and average (test for interaction), as described by Altman.\(^1\) Values of \( P < 0.05 \) were considered to be statistically significant.

Additionally, in the evaluation of the method to determine unidirectional permeability, Friedman’s rank sum test\(^1\) was used to assess any dependence on time of the 7, 8, 9, and 10 hours’ \( P^{\text{uni}} \) measurements during the two treatment periods.

### Subjects

Fifteen subjects gave their informed consent to participate in the study. Enrollment required the subjects to be in good health without known systemic illness including diabetes, hypertension (blood pressure more than 160/90 mm Hg), pregnancy, multiple allergies or adverse reactions to fluorescein or sulfa drugs, prior episode of kidney stones, or medication with diuretics. Furthermore, participants were excluded if they had had eye surgery or had any current eye disease, including significant cataract. Visual acuity had to be normal and any refractive anomaly had to be less than \( \pm 5 \) D.

The subjects underwent an ordinary eye examination with slit-lamp biomicroscopy, ophthalmoscopy, tonometry, and fundus color photography (60°). One participant was excluded after the first treatment period due to a moderate adverse reaction to fluorescein injection. The remaining 14 subjects included 8 women and 6 men with an average age of 22.9 years (range, 20–26 years).

### Results

In the calculation of \( P^{\text{uni}} \), the average D for the two treatment periods is used to limit uncertainty from the D-value measurements. The average D value was \( 5.4 \times 10^{-9} \pm 1.0 \times 10^{-6} \) cm\(^2\)/s (mean \( \pm \)SD; range, 3.9–7.2 \( \times 10^{-6} \) cm\(^2\)/s). During treatment with AZM and placebo, the D values were \( 5.5 \times 10^{-6} \pm 1.1 \times 10^{-6} \) cm\(^2\)/s (mean \( \pm \)SD; range, 4.2–7.5 \( \times 10^{-6} \) cm\(^2\)/s), and \( 5.3 \times 10^{-6} \pm 0.9 \times 10^{-6} \) cm\(^2\)/s (mean \( \pm \)SD; range, 3.9–7.2 \( \times 10^{-6} \) cm\(^2\)/s), respectively. \( P^{\text{uni}} \) is calculated as an average of values determined at 7, 8, 9, and 10 hours. Analysis of the \( P^{\text{uni}} \) values at 7, 8, 9, and 10 hours showed no statistically significant time dependence for placebo or AZM treatment (\( P = 0.061 \) and \( P = 0.28 \), respectively).

### Effects of AZM on \( P^{\text{pas}} \) and \( P^{\text{uni}} \)

For each patient, the values of \( P^{\text{pas}} \) and \( P^{\text{uni}} \) after treatment with AZM and placebo, respectively, are shown in Table 1. For \( P^{\text{pas}} \) there was no statistically significant period effect or treatment-period interaction (\( P = 0.61 \) and \( P = 0.097 \), respec-
Effects of AZM on Active and Passive Transport

**TABLE 1. Passive and Unidirectional Permeabilities during Treatment with Placebo and Acetazolamide**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>P(pas), nm/s</th>
<th>AZM P(pas), nm/s</th>
<th>P(uni), nm/s</th>
<th>AZM P(uni), nm/s</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1.55</td>
<td>1.95</td>
<td>38.6</td>
<td>37.1</td>
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<tr>
<td>2</td>
<td>1.79</td>
<td>2.00</td>
<td>22.7</td>
<td>32.0</td>
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<tr>
<td>3</td>
<td>2.03</td>
<td>2.60</td>
<td>46.8</td>
<td>56.2</td>
</tr>
<tr>
<td>4</td>
<td>1.79</td>
<td>2.21</td>
<td>17.9</td>
<td>26.3</td>
</tr>
<tr>
<td>5</td>
<td>1.99</td>
<td>3.01</td>
<td>37.9</td>
<td>54.1</td>
</tr>
<tr>
<td>6</td>
<td>1.73</td>
<td>2.14</td>
<td>-1.7</td>
<td>12.6</td>
</tr>
<tr>
<td>7</td>
<td>2.80</td>
<td>2.73</td>
<td>38.7</td>
<td>54.6</td>
</tr>
<tr>
<td>8</td>
<td>1.67</td>
<td>1.77</td>
<td>16.0</td>
<td>20.9</td>
</tr>
<tr>
<td>9</td>
<td>1.88</td>
<td>2.26</td>
<td>15.2</td>
<td>34.2</td>
</tr>
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<td>10</td>
<td>3.08</td>
<td>2.32</td>
<td>16.0</td>
<td>18.4</td>
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<td>2.17</td>
<td>2.68</td>
<td>29.3</td>
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<td>2.59</td>
<td>3.50</td>
<td>37.2</td>
<td>39.6</td>
</tr>
<tr>
<td>14</td>
<td>1.53</td>
<td>2.07</td>
<td>18.6</td>
<td>21.5</td>
</tr>
<tr>
<td>15</td>
<td>2.02</td>
<td>2.34</td>
<td>25.4</td>
<td>32.8</td>
</tr>
<tr>
<td>Avg</td>
<td>4.73</td>
<td>5.93</td>
<td>29.4</td>
<td>36.6</td>
</tr>
<tr>
<td>SD</td>
<td>0.48</td>
<td>0.46</td>
<td>13.2</td>
<td>14.1</td>
</tr>
</tbody>
</table>

P, placebo. P(pas) and P(uni) were calculated as average of the right and left eye. Subject no. 5 was excluded following the first examination due to suspected allergy towards fluorescein.

The present study of the pharmacological effect of the carbon anhydrase inhibitor AZM’s influence on the blood-retina barrier indicates that AZM stimulates the active transport of fluorescein and increases the passive permeability in healthy subjects. The interpretation of these results necessitates considerations of possible AZM effects on the vitreous pH and thereby fluorescence and dissociation of the fluorophores and on the electrochemical gradient. These considerations follow below. The implications of the results for the understanding of the edema-reducing effect of AZM are debated thereafter.

**Discussion**

The present study of the pharmacological effect of the carbon anhydrase inhibitor AZM’s influence on the blood-retina barrier indicates that AZM stimulates the active transport of fluorescein and increases the passive permeability in healthy subjects. The interpretation of these results necessitates considerations of possible AZM effects on the vitreous pH and thereby fluorescence and dissociation of the fluorophores and on the electrochemical gradient. These considerations follow below. The implications of the results for the understanding of the edema-reducing effect of AZM are debated thereafter.

**Effect of pH on the Dissociation of Fluorescein**

The calculations of the unidirectional permeability assume a uniform passive permeability in the directions vitreous to blood and blood to vitreous. A selective increase in the outward passive permeability from vitreous to blood could cause an overestimation of the unidirectional permeability. We therefore considered whether the increase in unidirectional permeability could be due to a pH-induced increase of the passive permeability in the direction vitreous to blood because a drop in the pH of the retina and vitreous has been seen in cats within the first hours after a bolus injection of AZM. Fluorescein can exist as a cation, neutral molecule, mono- and dianion with pK\textsubscript{a} values of 6.7, 4.4, and 2.2. At physiological pH, fluorescein is found as mono- and dianion, and a drop in pH, which increases the proportion of the monoanion form, will lead to increased lipid solubility. However, studies indicate that fluorescein passes the blood-retina barrier through water-filled pores and a change in fluorescein’s lipid solubility caused by a decline in pH is therefore unlikely to affect the results significantly.

**Effect of pH on Fluorescence**

Because the fluorescence of fluorescein decreases significantly even with minor reductions in pH, a decline in the pH of the vitreous would cause an underestimation of the vitreous concentrations of fluorescein. An underestimation of the vitreous concentration of fluorescein would cause an overestimation of the unidirectional permeability (and underestimation of passive permeability). A pH decline of the magnitude observed in cats (0.14 pH U), would seem unable to explain the increase in unidirectional permeability of 29% found in this study as appears from the following. Fluorescence coefficients established from measurements of fluorescein standards at physiological pH (pH 7.34) are used for the determination of fluorescein concentrations in the blood and vitreous. A previous study examined fluorescence intensities at varying pHs (interval 6.5-7.5) from a 1 µM fluorescein solution and calculation of the apparent fluorescein concentration based on fluorescence intensity measurements and fluorescence coefficients determined at physiological pH. The apparent concentration as a function of pH is approximately linear, and based on the linear regression output (slope, 0.046 µM/pH U; constant, -0.024 µM) it can be calculated that a drop of 0.14 pH U compared to physiological pH leads to an underestimation of the fluorescein concentration of approximately 6%, which will lead to an overestimation of P(uni) of roughly the same magnitude. Previous studies have been limited to the study of the acute effects of AZM on retinal and vitreous pH, and it is possible that pH is normalized after some time due to mechanisms that counter this immediate effect of AZM.

**Electrochemical Forces**

We also considered whether AZM-induced changes of the electrochemical forces could play a role. The fluorometric calculations assume the same potential on both sides of the blood-retina barrier. Acetazolamide given IV is followed immediately by a decline in the electrochemical potential of the eye. However, the effect seems to be relatively brief because the electrochemical forces in the eye assessed from an electrooculogram in healthy subjects is approximately 0.046 µM/pH U; constant, -0.024 µM) it can be calculated that a drop of 0.14 pH U compared to physiological pH leads to an underestimation of the fluorescein concentration of approximately 6%, which will lead to an overestimation of P(uni) of roughly the same magnitude. Previous studies have been limited to the study of the acute effects of AZM on retinal and vitreous pH, and it is possible that pH is normalized after some time due to mechanisms that counter this immediate effect of AZM.

**Considerations of the AZM Effect on Passive Transport**

We found an increase in passive permeability during AZM treatment. A previous study of leakage assessed from the flu-
The increase in passive permeability in healthy subjects in the present study contrasts with the statistically significant decrease in passive permeability found in a study of 7 patients with retinitis pigmentosa and a decrease in the posterior penetration ratio in a study of 30 patients with chronic iridocyclitis complicated by macular edema after 2-week and 1-month tablet treatments with AZM, respectively.4,5

We have no solid explanation for the increase in $P^{(pas)}$. Based on the previous studies, a limited decline or no change in $P^{(pas)}$ would have been expected. It was considered whether the increase in $P^{(pas)}$ could have been related to capillary dilation caused by an AZM-induced increase in the blood's content of CO$_2$, PaCO$_2$. Inhibition of carbonic anhydrase leads to increased loss of HCO$_3^-$ in the kidneys and to metabolic acidosis in consequence. The metabolic acidosis is compensated for by increased ventilation, and a study of healthy subjects using the same daily doses of AZM as in the present study showed a reduced PaCO$_2$ after 4 days of medication.25 Changes in the blood's acid-base status can therefore hardly explain the increase in passive permeability.

An AZM-induced dilation of arterioles related to a reduced pH in the retinal extracellular fluid has been proposed.26 It could be speculated that an increased capillary area (recruitment of capillaries) could be an explanation for the observed increase in $P^{(pas)}$. Such an effect may be largest in healthy subjects, probably those with the highest metabolic activity and H$^+$ production. Assuming that the retinal pH gradually normalizes over time, the shorter treatment time of this study could have caused a relatively lower retinal pH compared with the cited studies.4,5

Apart from the apical and basolateral membranes of the pigment epithelium, carbonic anhydrase is localized to the endothelium of the retinal capillaries, the Müller cells, and outer segment of the cones.27,28 A final speculation could be that the difference in AZM's effect on $P^{(pas)}$ is due to a different effect on the barrier cells, causing shrinkage or swelling, respectively, depending on which side of the barrier is affected, implying that AZM's effect depends on the extent to which it can penetrate the blood-retina barrier. There exists no experimental data to clarify this matter.

Effect on $P^{(uni)}$ and the Implications for the Edema-Reducing Effect of AZM. The increase in unidirectional permeability confirms the results from our study of AZM’s effect on active transport of fluorescein in seven patients with retinitis pigmentosa, five of whom had varying degrees of angiographic macular leakage.32 That study showed evidence that AZM affects the blood-retina barrier cells with stimulation of at least one pump mechanism. It may be possible that other transport systems are affected, but this cannot be determined by the present study, which was limited to quantification of the transport of fluorescein. It is not known which transport mechanism is responsible for the active transport of fluorescein.

It has been proposed that the edema-reducing effect of AZM is due to a stimulation of ion and fluid removal.3 A stimulated ion and fluid removal has also been proposed on the basis of experiments in animals.29,30 Animal experiments suggest that the pigment epithelium has a considerable potential for drawing fluids from the retina to the choroid by means of ion transport.31 The transport mechanism for water across the blood-retina barrier remains incompletely understood. A recent study suggests cotransport of lactate and water across the pigment epithelium.52 It is unknown whether other cotransport systems for water are attached to the blood-retina barrier and whether the transport mechanism for fluorescein is involved. Future studies could clarify this.

Methodological Issues

The method used to determine the active component was with some modification identical to that described by Engler et al.9 As stated in the Methods section, calculation of the active transport involves the determination of the preretinal gradient from 7 to 10 hours. Compared to Engler et al. we used fluorescence data in a slightly larger distance from the retina (1–5 mm rather than 0.5–5 mm from the retina) and used linear rather than quadratic fitting for the estimation of the preretinal gradient. The purpose in using fluorescence data at a larger distance was to ensure that all data points referred to the vitreous despite small differences in alignment and to minimize the range of the fluorescence from the retina. Linear fit was used instead of quadratic fit because preretinal curves did not indicate any significant bend toward the retina. To obtain adequate precision in the determination of the permeabilities, several scans are required. For the determination of $P^{(pas)}$, six scans were recorded, and for the determination of $P^{(uni)}$ 16 scans were recorded. The high number of scans used to determine $P^{(uni)}$ was due to the low concentrations of fluorescein in the blood and in the vitreous, causing a relatively higher measurement uncertainty. In the original methodological work, hourly measurements from 7 to 12 hours after fluorescein injection were used.9 In the present study the measurement period was limited to 10 hours because in our experience vitreous measurements in healthy subjects performed after 10 hours are usually affected by significant noise. There was no statistically significant time dependence.

Earlier studies use the terminology “outward permeability” and “inward permeability.” The term “inward permeability” is susceptible to misinterpretation and corresponds to passive permeability used in this study. “Outward permeability” comprises both the active and passive components. For Table 2 unidirectional permeability for previously published data were calculated by deducting passive permeability from outward permeability. The comparison of previously published data for healthy subjects with the placebo values of this study show a better agreement for passive permeability than for unidirectional permeability.7–9 In all studies $P^{(uni)}$ is above zero in accordance with the existence of an active transport.

Most studies7–9 have been based on the measurement of fluorescence that did not permit any distinction between the

<table>
<thead>
<tr>
<th>Author</th>
<th>$P^{(pas)}$</th>
<th>$P^{(uni)}$</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeimer et al.8</td>
<td>1.2</td>
<td>34</td>
<td>19</td>
</tr>
<tr>
<td>Ogura et al.7</td>
<td>3.0</td>
<td>90</td>
<td>13</td>
</tr>
<tr>
<td>Engler et al.9</td>
<td>1.4</td>
<td>150</td>
<td>5</td>
</tr>
<tr>
<td>This study (placebo results)</td>
<td>2.0</td>
<td>25.4</td>
<td>14</td>
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$P^{(uni)}$ is calculated by deducting passive permeability from outward permeability.
specific contributions of fluorescein and fluorescein glucuronide. Nevertheless, the unidirectional permeabilities in the present study differed only slightly from the values found by Zeimer et al. using a computer simulation technique and applying a modified version of a mathematical model developed by Palestine et al. In a smaller study, Engler et al. found values of unidirectional permeability that were a factor of six higher than the placebo results in the present study. To analyze this difference further we recalculated the unidirectional permeability during placebo treatment in the present study on the basis of quadratic fitting of fluorescence data recorded 0.5 to 5 mm from the retina as used by Engler et al. The use of the calculation methodology of Engler et al. resulted in a unidirectional permeability during placebo treatment in this study of 95.2 nm/s, which reduced the difference between the present study and the study of Engler et al. to 58%. Coauthors Lund-Andersen and Sander state that technical problems with the laser light source used in the study by Engler et al. may have contributed to the remaining difference. As discussed above we consider the method presented in this study to be preferable.

Acknowledgments

Diamox and placebo tablets were generously supplied by Lederle Inc. The authors thank Hans Henrik Petersen for photographic assistance, Professor Ove Sten-Knudsen, Associate Professor Jesper Brah, and Morten La Cour for valuable comments; and Associate Professor Peter Dalgaard, PhD, MSc, for comments on the statistical analysis.

References


Effect of Acetazolamide on BRB Transport of Fluorescein 1775