Effects of Pilocarpine and Tropicamide on Blood-Aqueous Barrier Permeability in Man

Mikiro Mori, Makoto Araie, Masahiko Sakurai, and Tersuro Oshika

The time courses of changes in the effects of topical pilocarpine and tropicamide on the index of the blood-aqueous barrier permeability to plasma protein (P_{in}) were determined in normal volunteers. Before and after drug instillation in one eye, protein concentration in the anterior chamber (C_{a}) was determined from aqueous flare intensity with a laser flare-cell meter and from aqueous flow by fluorophotometry. The P_{in} was calculated from the C_{a}, plasma protein concentration, and aqueous flow. One percent pilocarpine produced a maximum increase of 21 \pm 10\% in the C_{a} (mean \pm SEM, n = 10), no significant change in the aqueous flow (n = 5), and a maximum increase of 29 \pm 10\% in the P_{in} (n = 10). Three percent pilocarpine produced a maximum increase of 55 \pm 11\% in the C_{a} (n = 8), a maximum increase of 34 \pm 13\% in the aqueous flow (n = 5), and a maximum increase of 74 \pm 18\% in the P_{in} (n = 8). Tropicamide (0.4\%) produced a maximum decrease of 17 \pm 7\% in the C_{a} (n = 8), a maximum decrease of 15 \pm 11\% in the aqueous flow (n = 8), and a maximum decrease of 24 \pm 13\% in the P_{in} (n = 8). The results indicated that pilocarpine increased the blood-aqueous barrier permeability to plasma protein in a dose-dependent manner and that tropicamide reduced it. Invest Ophthalmol Vis Sci 33:416-423, 1992

Pilocarpine, a muscarinic agonist, has been in clinical use for more than 100 years.\(^1\) This drug is still one of the mainstays for the medical management of glaucoma,\(^1\) whereas its use is contraindicated in inflamed or neovascularized eyes.\(^1\) Wessely first reported that topical pilocarpine increases proteins in the aqueous,\(^4\) and several studies have shown that it increases the permeability of the blood-aqueous barrier (BAB) in animals.\(^5\) In humans, a preoperative use of pilocarpine is associated with more frequent and severe postoperative complications,\(^5,6\) and stronger miotics such as anticholinesterase agents cause apparent inflammation in the anterior segments of the eye.\(^10\) In contrast, atropine, a muscarinic antagonist, is reported to reduce the BAB permeability in animals\(^6,11\) and is considered to moderate inflammation in human eyes.\(^10\) However, the effects of these drugs on the BAB permeability have not been well determined in human eyes despite their clinical importance. Reports are limited to fluorophotometric studies, which showed that topical pilocarpine has little effect on the BAB permeability to fluorescein in human eyes.\(^12,13\) No studies have been made on the effects of muscarinic antagonists.

Fluorophotometric methods of evaluating the BAB permeability have the following problems: (1) the clinically significant barrier function of the BAB cannot be evaluated by its permeability to fluorescein, an exogenous substance whose molecular weight is the order of 1/100 of that of plasma proteins; (2) systemically administered fluorescein is metabolized into a substance with different fluorescence, making quantitative analyses unreliable;\(^14,15\) and (3) transient changes in the BAB permeability may not be detected. Therefore, a method of directly monitoring the BAB permeability to plasma protein is more desirable than fluorophotometric methods.

A previous study suggested that the BAB permeability to plasma protein can be monitored by determining the protein concentration in the anterior chamber and the aqueous flow rate simultaneously.\(^16\) However, this method has not been used because the protein concentration in the anterior chamber was difficult to continually determine in living eyes. Recently developed instruments have made such determination easy and accurate,\(^17,18\) and the sensitivity of these instruments in evaluating the aqueous flow rate has been confirmed.\(^18-20\) In the present study, we used this method to evaluate the effects of topical pilocarpine and tropicamide on the BAB permeability in human eyes and found that topical pilocarpine increased...
the BAB permeability, while topical tropicamide reduced it.

Materials and Methods

Subjects

Twenty six normal volunteers aged 20–27 years participated in the present study. All had dark brown irides. Informed written consent was obtained from each subject after the procedure and the drugs had been fully explained. This study was approved by the Ethics Committee of the University of Tokyo School of Medicine.

Drugs

Ophthalmic solutions of 1% and 3% pilocarpine hydrochloride (Sanpilo 1% and 3%) and 0.4% tropicamide (Mydrin-M), and the vehicle solutions were supplied by Santen Pharmaceutical (Osaka, Japan) from a recently manufactured stock.

Measurement of Flare Intensity in the Anterior Chamber

Flare intensity in the anterior chamber was measured with a laser flare-cell meter (FC-1000; Kowa, Tokyo, Japan). Recorded flare intensity (photon count/ms) was converted into the equivalent of human albumin concentration (mg/100ml), as previously described.\textsuperscript{19,20}

Pilocarpine: Ten and eight subjects were studied with 1% and 3% pilocarpine, respectively. Flare measurements were made from 9 a.m. to 9 p.m. at half-hour to two-hour intervals. Intraocular pressure (IOP) was measured with an applanation tonometer, and pupil diameter was measured with a caliper under constant illumination after each flare measurement. At 11 a.m., 30 \( \mu l \) of 1% or 3% pilocarpine was instilled in one randomly chosen eye, and the vehicle was instilled in the fellow eye. Venous blood samples were collected at 5 p.m., and total plasma protein concentration was determined by the biuret test.

Tropicamide: Eight subjects were studied. Measurements of flare intensity, IOP, and pupil diameter were made from 10 a.m. to 5 p.m. at half-hour intervals. At 11 a.m., 30 \( \mu l \) of 0.4% tropicamide was instilled in one randomly chosen eye, and the vehicle was instilled in the fellow eye. Venous blood samples were collected at 5 p.m., and total plasma protein concentration was determined by the biuret test.

Determination of Aqueous Flow Rate

One week after the flare measurements, fluorophotometry was carried out with a commercially avail-

\[ f(t) = 0.9 \left[ V_c \frac{dF_c(t)}{dt} + V_a(t)\frac{dF_a(t)}{dt}\right]/F_a(t) \]

where \( V_c \) is the volume of the corneal stroma, which was assumed to be 70 \( \mu l \),\textsuperscript{24} \( F_a(t) \) is the fluorescein concentration in the corneal stroma at time \( t \), \( F_c(t) \) is the fluorescein concentration in the anterior chamber at time \( t \), and 0.9 is the correction factor for diffusional
loss of fluorescein across the iris surface. The terms \( \frac{dF_c(t)}{dt} \) and \( \frac{dF_a(t)}{dt} \) were approximated with the difference equations.

Calculation of Index of BAB Permeability to Protein

Because protein leaves the anterior chamber exclusively by the bulk aqueous flow, the dynamics of protein transfer in the anterior chamber is given by:

\[
\frac{dM_a(t)}{dt} = P_{in}(t)[C_p - C_a(t)] - f(t)C_a(t) \quad (2)
\]

where \( M_a(t) \) is the mass of protein in the anterior chamber at time \( t \), \( C_a(t) \) is the protein concentration in the anterior chamber at time \( t \), \( C_p \) is the total plasma protein concentration, which was assumed to be constant during the experiment, and \( P_{in}(t) \) is the index of BAB permeability to plasma protein at time \( t \). Because \( M_a(t) \) is the product of \( C_a(t) \) and the anterior chamber volume at time \( t \), \( V_a(t) \), equation 1 is rewritten as:

\[
P_{in}(t) = \left[ f(t)C_a(t) + \frac{dV_a(t)}{dt}C_a(t) \right. \\
\left. + \frac{dC_a(t)}{dt}V_a(t) \right]/(C_p - C_a(t)). \quad (3)
\]

In calculating the \( P_{in}(t) \), individual values of \( f(t) \) and \( V_a(t) \) were used for those subjects who participated in the flare measurements and fluorophotometric experiments. For those who participated in only the flare measurements, the average values of \( f(t) \) and \( V_a(t) \) obtained in those who participated in both of the experiments were used. The terms \( dC_a(t)/dt \) and \( dV_a(t)/dt \) were approximated with the difference equations.

In all of the above experiments, the measurements obtained in the fellow untreated eye served as the baseline data for those obtained in the treated eye.

Results

Figures 1–3 show the time courses of changes in \( C_a \), pupil diameter, aqueous flow rate, IOP, and \( V_a \) before and after instillation of each drug. In all subjects, apparent fluorescein concentration in the cornea was higher than 700 ng/ml at the beginning of the fluorophotometric experiments. This was high enough for fluorophotometry to be conducted with adequate precision throughout the experimental periods.

1% Pilocarpine

The \( C_a \) increased significantly at 3, 8, and 10 h after instillation with a maximum increase of 21 ± 10% at 8 h (mean ± SEM, \( n = 10 \), \( P < 0.05 \), paired t-test). The pupil diameter decreased significantly between 0.5 and 10 h with a maximum decrease of 2.0 ± 0.2 mm at 2 h. The IOP was significantly reduced at 1–3 h with a peak reduction of 1.4 ± 0.5 mmHg at 2 h. Pupil diameter and IOP on the day of the fluorophotometric experiment (filled triangles, treated eyes; open triangles, fellow control eyes) did not differ significantly (\( P > 0.1 \), unpaired t-test) from those seen on the day of the flare measurements (filled and open circles).
3% Pilocarpine

The \( C_a \) increased significantly at 1–4 and 6 h after the instillation with a maximum increase of 55 \pm 11\% at 1 h (mean \pm SEM, \( n = 8 \), \( P < 0.05 \), paired t-test). The pupil diameter decreased significantly between 1 and 10 h with a maximum decrease of 3.4 \pm 0.2 \text{ mm}.
at 2 h. The IOP decreased significantly at 1-10 h with a peak reduction of 2.0 ± 0.5 mmHg at 3 h. The pupil diameter and IOP on the day of the fluorophotometric experiment \((n = 5)\) showed changes similar to those seen on the day of the flare measurements \((P > 0.1,\) unpaired t-test). The aqueous flow rate increased significantly at 4, 5, 8 and 10 h with a maximum increase of 34 ± 13% at 5 h \((n = 5)\). The \(V_a\) decreased significantly between 1 and 6 h with a maximum decrease of 10 ± 2% at 2 h. The \(C_p\) was 7.2 ± 0.2 g/100 ml \((n = 8)\).

### 0.4% Tropicamide

The \(C_a\) decreased significantly 1 and 1.5 h after the instillation with a maximum decrease of 17 ± 7% at 1.5 h \((\text{mean ± SEM, } n = 8, \ P < 0.05, \text{ paired t-test})\). The pupil diameter increased significantly between 0.5 and 5 h with a maximum increase of 3.6 ± 0.1 mm at 1 h. The IOP did not change significantly throughout the experimental period \((P > 0.2, \ n = 8, \text{ paired t-test})\). The pupil diameter and IOP on the day of the fluorophotometric experiment showed changes similar to those observed on the day of the flare measurements \((P > 0.1, \ n = 8, \text{ paired t-test})\). The aqueous flow rate decreased significantly at 4 and 5 h with a maximum decrease of 15 ± 11% at 5 h \((n = 8)\). The \(V_a\) increased significantly between 1 and 4 h with a maximum increase of 12 ± 4% at 2 h. The \(C_p\) was 7.1 ± 0.3 g/100 ml \((n = 8)\).

Figure 4 shows the time course of changes in the index of the BAB permeability to plasma protein before and after drug instillation. The index of the BAB permeability increased significantly at 4 and 5 h after the instillation of 1% pilocarpine with a maximum increase of 29 ± 10% at 4 h \((\text{mean ± SEM, } n = 10, \ P < 0.05, \text{ paired t-test})\). It also increased at 1 h and 3-6 h after the instillation of 3% pilocarpine with a maximum increase of 74 ± 18% at 1 h \((n = 8)\). It decreased significantly 4 h after the instillation of 0.4% tropicamide by 24 ± 13\% \((n = 8)\). Figure 5 shows the time course of changes in the ratio of the index of the BAB permeability in the treated eye to that in the fellow control eye before and after the instillation of each drug. The ratio of the index in the 3% pilocarpine-treated subject was significantly higher than that in the 1% pilocarpine-treated subject at 1 and 4 h after instillation \((P < 0.05, \text{ unpaired t-test})\).

### Discussion

The index of the BAB permeability to plasma protein is most accurately determined by simultaneous measurements of the flare intensity and aqueous flow rate. However, we made flare and fluorophotometric measurements on two separate days to avoid the influence of highly concentrated fluorescein in the cornea on the measurements of glare intensity in the anterior chamber. Furthermore, making all the above measurements simultaneously at 0.5- to 1-h intervals was difficult because of their time requirements. The effects of the drugs were probably the same in the two experiments because the values of IOP and pupil di-
ameter in both eyes on the day of the fluorophotometric experiment were close to those on the day of flare measurements at each corresponding point of measurement.

Because some of the subjects did not participate in the fluorophotometric experiments with 1% and 3% pilocarpine, we calculated $P_{in}(t)$ for them using the averaged values of $f(t)$ and $V_a(t)$ obtained in those who participated in both of the experiments. If we calculate $P_{in}(t)$ only from the data of the five subjects who participated in both of the experiments, the increase in $P_{in}(t)$ after the instillation of 1% pilocarpine is significant at 4 and 5 h ($P < 0.05$) and the maximum increase is $31 \pm 15\%$ at 4 h. In addition, the increase in $P_{in}(t)$ after the instillation of 3% pilocarpine is significant at 1 h and 3–6 h ($P < 0.05$) and the maximum increase is $80 \pm 27\%$ at 1 h. These figures are close to those of the results we obtained above, indicating that no serious errors arise from the calculation of $P_{in}(t)$ using the averaged values of $f(t)$ and $V_a(t)$ in some subjects.

Pilocarpine increased the flare intensity in the anterior chamber and tropicamide decreased it. Miosis probably did not affect the flare measurement, based on the following reasons. First, Figure 1 shows that the increase in the flare intensity does not parallel the decrease in the pupil diameter. Second, Figure 2 shows that the flare intensity in the 3% pilocarpine-treated eye at noon was much higher than that at 1 p.m., while the pupil diameter was almost the same at the two points. Small pupil reduces the area where the sampling window can be positioned without increasing background scatter, leading to difficulty with flare measurement, particularly in elderly subjects. In such a case, the instrument discards measurement data more frequently because of high background signal intensity. The difficulty is minimized when subjects have clear lenses and are cooperative enough to allow careful positioning of the sampling window.

In addition, it is also unlikely that mydriasis lowered the protein concentration in the anterior chamber by mixing the anterior aqueous with the posterior aqueous containing less protein because no significant increase in the rate of fluorescein loss from the anterior chamber was found after mydriasis by tropicamide, as shown in Figure 6. This rate would be increased if mydriasis had caused mixture of the anterior and posterior aqueous, because the fluorescein concentration in the posterior aqueous should be much lower than that in the anterior aqueous. Similar findings were reported in a previous fluorophotometric study.

A single instillation of 3% pilocarpine increased the aqueous flow rate, while a single instillation of 1% pilocarpine did not. A previous study in whites by the topical fluorescein method showed that a single instillation of 0.5% pilocarpine increased the aqueous flow rate. The discrepancy may be ascribed to the difference in uveal pigmentation between the two subject groups, although the sample size of the present study is small. In contrast, topical tropicamide reduced the aqueous flow rate. Considering that muscarinic receptors are related to the intracellular cyclic-AMP levels and that cyclic-AMP levels affect aqueous humor formation, the present results—i.e., an increase in the aqueous flow rate after a muscarinic agonist and a decrease after an antagonist—suggest that aqueous humor formation in human eyes is at least partly regulated through muscarinic receptors.

The present results show that pilocarpine increased the index of the BAB permeability to protein in a dose-dependent manner and that tropicamide reduced it. Here, the effect of the BAB permeability on the aqueous flow determined fluorophotometrically must be considered, because it leads to an overesti-
mate of the changes in the $P_{in}(t)$ calculated with equation 3. The aqueous flow rate, which we calculated with equation 1, is more precisely given as:

$$f(t) = \left(1 - \frac{k_{dpa}(t)}{k_{out}(t)}\right) \int \frac{dF_{e}(t)}{dt} + \frac{dV_{s}(t)F_{e}(t)}{dt} \right] / F_{e}(t) \tag{4}$$

where $k_{dpa}(t)$ is the coefficient of fluorescein transfer by diffusion at time $t$ and $k_{out}(t)$ is the coefficient of total fluorescein loss from the anterior chamber at time $t$. Because the change in $k_{dpa}(t)$ is unknown in the present study, it is assumed that the change in $k_{dpa}(t)$ $V_{s}(t)$—the index of BAB permeability to fluorescein—parallels that in $P_{in}(t)$, the index of BAB permeability to protein [ie, $k_{dpa}(t) V_{s}(t) = \alpha P_{in}(t)$]. $P_{in}(t)$ and $f(t)$ are then rewritten as:

$$P_{in}(t) = \left[\int f^*(t)C_{a}(t) + \frac{dV_{s}(t)}{dt} C_{a}(t) \right.\
\left. + \frac{dC_{a}(t)}{dt} V_{s}(t)\right] / \left[C_{p} - (1 - \alpha)C_{a}(t)\right] \tag{5}$$

$$f(t) = f^*(t) - \alpha P_{in}(t) \tag{6}$$

where $\alpha$ is a constant, and

$$f^*(t) = \left[\int V_{c} \frac{dF_{e}(t)}{dt} + \frac{dV_{s}(t)F_{e}(t)}{dt} \right] / F_{e}(t). \tag{7}$$

Because $k_{dpa}(t)$ in normal eyes is reported to be in the order of $1 \times 10^{-3}$/min, $\alpha$ is calculated to be about 20, using the average values of $C_{a}(t)$, $C_{pp}$, $V_{s}(t)$, and $f(t)$ for the untreated eyes in the present study. The maximum changes in $P_{in}(t)$ recalculated with the above equations are 29 ± 11% (mean ± SEM, $n = 10$), 69 ± 15% ($n = 8$), and 23 ± 13% ($n = 8$) for 1% pilocarpine, 3% pilocarpine, and 0.4% tropicamide, respectively. These figures are close to those of the present results obtained with equations 1 and 3, indicating that the calculation of $P_{in}(t)$ without correcting $f(t)$ for the changes in the BAB permeability produces no substantial errors. Regarding aqueous flow, the $f(t)$ corrected for the increase in the BAB permeability using equation 6 is 4% smaller than the uncorrected $f(t)$ 1 h after the instillation of 3% pilocarpine, when the change in the index of the BAB permeability was at its maximum. This indicates that the calculation of $f(t)$ without correction for the changes in the BAB permeability also produces no substantial errors. Although little is known about the exact mechanism or mechanisms underlying the increase in the index of the BAB permeability by muscarinic agonists, it may be explained by: (1) an increase in the permeability of the ciliary epithelium; (2) an increase in the endothelial permeability of the vessels in the ciliary processes and their iridial extensions; or (3) an increase in the blood flow through the anterior uvea. The decrease in $P_{in}(t)$ after tropicamide may be explained by the effects contrary to those of pilocarpine, although its mechanisms also are unclear.

Protein entry into the anterior chamber also may be affected by ciliary muscle contraction and relaxation. Under normal conditions, plasma protein molecules are thought to leak out of the capillaries in the ciliary body and diffuse through the ciliary and iris stroma into the anterior chamber, being the source of proteins in the anterior chamber. Because some of the proteins in the ciliary process stroma are thought to diffuse through the ciliary muscle and leave the eye through the sclera, ciliary muscle contraction may reduce the loss of protein through this route and increase protein entry into the anterior chamber. On the other hand, ciliary muscle contraction should restrict the movement of protein molecules in the stroma as well as the movement of water molecules, the uveoscleral flow, in order to decrease in protein entry. Converse mechanisms are assumed to hold in the case of ciliary muscle relaxation. The total effect of ciliary muscle contraction or relaxation on the protein entry by diffusion may be slight because there are factors offsetting each other. Another possible effect of ciliary muscle contraction on protein entry is a release of proteins stored in the iris-ciliary stroma. The sharp rise in the protein concentration in the anterior chamber found immediately after the instillation of 3% pilocarpine may have been influenced by this effect. However, it is not reasonable to attribute the continuous increase in $P_{in}(t)$ in the present study to this effect alone, which cannot last long after drug instillation.

The equation we used to describe the protein dynamics in the anterior chamber does not include a term accounting for a time delay in protein entry that results from passage through the iris-ciliary stroma. Such a delay was quantified in the previous study using a computational model to simulate the steady-state protein diffusion through the iris and process stroma in rabbits. This model, when incorporated in the present analyses, may help determine the fraction of the change in $P_{in}(t)$ that results from the change in protein diffusion through the iris-ciliary stroma with no net changes in the permeability of the ciliary epithelium and iris vessel endothelium. To apply the model in the present study, however, further investigations are needed to reveal how pilocarpine and tropicamide change the model parameters for human eyes, eg, the size and tissue porosity of the iris ciliary stroma, the protein diffusivity, and the constant of transcapillary protein flow.

The $P_{in}(t)$ calculated in the present study represents the permeability of the BAB in a broad sense, quantifying the ease with which plasma proteins enter the anterior chamber. Nevertheless, clinical implications of $P_{in}(t)$'s changes deserve discussion. We found that a single instillation of pilocarpine in the eyes of young...
normals significantly increased the BAB permeability. The increase is probably more pronounced in aged patients undergoing long-term pilocarpine therapy because the integrity of the BAB may deteriorate with aging. Thus, care must be taken in the surgical management of these patients. On the other hand, postoperative atropinization has been thought to be important not only for dilating the pupil and relaxing the ciliary body but for reducing the BAB permeability. The present results also offer an experimental basis for the above idea by showing that topical tropicamide reduces the BAB permeability in human eyes.

Key words: pilocarpine, tropicamide, blood-aqueous barrier permeability, protein concentration in the anterior chamber, aqueous flare

References


