Effect of Two Weeks of Timolol Maleate Treatment on the Normal Retinal Circulation

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The effect of 2 weeks of topical treatment with timolol maleate 0.5% ophthalmic solution on the retinal circulation was investigated using bidirectional laser Doppler velocimetry and monochromatic fundus photography. Fifteen normal healthy volunteers were included in this study. In a double-masked, randomized design one eye of each received one drop of timolol maleate 0.5% twice daily for 14 days, and the fellow eye received placebo. Vessel diameter (D), maximum erythrocyte velocity (V\text{max}), and volumetric blood flow rate (Q) were determined in one major retinal vein of each eye before treatment, and then, 2 hr after the instillation of drops on the morning of the 15th day of treatment. After treatment, the average change from baseline in D (+0.7%) V\text{max} (−8.2%) and Q (−7.7%) were not statistically significant in the placebo-treated eyes. In the timolol-treated eyes, the average increase from baseline in D (+0.1%) and Q (+10%) were not statistically significant. Average V\text{max} on the other hand, increased significantly from baseline (P < 0.05) by 9.6%. In comparison to the placebo-treated eyes, V\text{max} and Q were significantly increased in the timolol-treated eyes (P < 0.0005 and P < 0.01, respectively). These results are similar to those reported previously in a study of the effect of a one-time instillation of timolol and, therefore, suggest that the effect of timolol on the retinal circulation is maintained over a 2-week period. Invest Ophthalmol Vis Sci 32:39–45, 1991

Previous studies using laser Doppler velocimetry and monochromatic fundus photography suggest that the instillation of topical timolol maleate produces an average increase in retinal volumetric blood flow of about 13% in normal eyes\textsuperscript{1} and about 8% in eyes with ocular hypertension.\textsuperscript{2} Such an increase may be beneficial in patients with increased intraocular pressure (IOP) and retinal vascular occlusion in which antiglaucomatous therapy is used.\textsuperscript{3} To investigate whether the effect on blood flow is maintained with more prolonged timolol therapy, bidirectional laser Doppler velocimetry (BLDV) and monochromatic fundus photography (MFP) were used to study the effect of timolol maleate 0.5% instilled twice daily for 2 weeks on the retinal circulation.

Materials and Methods

Fifteen healthy volunteers aged 21–43 years (mean \pm 1 standard deviation, 30 \pm 7 years) with no history of systemic or intraocular disease were included in this study. Corrected visual acuities were 6/7.5 or better, and IOPs were 19 mmHg or below. Slit-lamp and funduscopic examinations were normal. None of these subjects were receiving topical or systemic medication at the time of the study. Informed consent was obtained from each subject after the procedures were explained.

After pupillary dilatation with tropicamide 1%, Polaroid (Cambridge, MA) color fundus photographs of the discs were taken. The BLDV measurements of the maximum or centerline erythrocyte velocity, V\text{max}, in a main superior or inferior temporal retinal vein were obtained in both eyes. These determinations were made in veins because the minimal flow pulsatility in these vessels simplifies the determination of the average velocity. The location of the measurement site was marked on the Polaroid photograph. Detailed descriptions of the BLDV technique and measurement procedure used in this study have been published before.\textsuperscript{1,2} Therefore, only a summary will be provided here.

Fundus photographs were taken in monochromatic light at 570 nm immediately after the BLDV recordings using a Zeiss fundus camera (Oberkochen, Germany) and Kodak Plus-X pan film (Rochester, NY). The diameter of veins (D) at the site of the BLDV recordings was measured from photographic negatives; D was obtained from an average of the diameters measured from six photographs. Fundus photography and BLDV determinations were done.

\textsuperscript{1} From the Scheie Eye Institute, Department of Ophthalmology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

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in a darkened room with the patient in a sitting position.

Retinal volumetric blood flow rate, Q, was calculated from the relation: 
\[ Q = \frac{V_{\text{mean}} \cdot \pi D^2}{4} \]
where \( V_{\text{mean}} \) represents the mean velocity of whole blood.
We assumed that \( V_{\text{mean}} = cV_{\text{max}} \), with \( c \) being a constant which is the same for all vessels measured. In this study, a value of \( c = 1/1.6 \) was adopted, based on the work of Damon et al., who studied the relationship between \( V_{\text{max}} \) and \( V_{\text{mean}} \) in glass tubes.

After baseline BLDV and MFP, the heart rate was determined, and systolic and diastolic brachial blood pressures were measured by sphygmomanometry. Two drops of topical proparacaine HCl 0.5% were instilled in each eye, and the IOP was measured by Goldmann applanation tonometry.

In a double-masked randomized design, one eye of each subject received one drop of timolol maleate 0.5% ophthalmic solution twice daily for 14 days; the fellow eye received placebo, consisting of the vehicle of the timolol ophthalmic solution. To assure compliance with the treatment protocol, IOP was monitored four or more times during the 14 days. On the morning of the 15th day, one drop of timolol was instilled in one eye and one drop of placebo, in the fellow eye. Two hours later, Q was measured again with the experimental procedure described.

Mean brachial artery blood pressure, \( B P_m \), was calculated according to the formula \( B P_m = B P_d + \frac{1}{2} (B P_s - B P_d) \), where \( BP_s \) and \( BP_d \) are the brachial artery systolic and diastolic pressures. Perfusion pressure, PP, was calculated as \( PP = \frac{1}{2} B P_m - IOP \).

All measurements of vessel diameter were done by one trained examiner, and all \( V_{\text{max}} \) determinations were done by another. Each individual was masked with regard to: (1) the results of the other, (2) whether measurements were obtained at baseline or after treatment, and (3) the eye that had received timolol.

Statistical evaluation of the data was performed using paired student t-test (two-tailed), linear regression, and correlation analysis. The presence of a normal distribution of the data was assessed by the Wilk-Shapiro normality test. Findings with an error probability value smaller than 0.05 were considered to be statistically significant.

**Results**

No significant change in heart rate or \( B P_m \) was observed after treatment. Average IOP decreased significantly by 23% in the timolol-treated eyes (paired t-test, \( P < 0.0001 \)) and insignificantly by 3.3% in the placebo-treated eyes (\( P > 0.05 \)). Perfusion pressure showed an insignificant average increase of 6.8% in the timolol-treated eyes (\( P > 0.05 \)) and an average 1.1% decrease in the placebo-treated eyes (\( P > 0.05 \)) (Tables 1 and 2). The IOPs measured in the timolol-treated eyes during the 14 days of the study were consistently lower than baseline.

Average values of \( D, V_{\text{max}}, \) and \( Q \) before and after 14 days of placebo and timolol treatment are summarized in Table 3. Average percentage change in \( D, V_{\text{max}}, \) and \( Q \) in the placebo- and timolol-treated eyes are shown in Figure 1.

After treatment, the average change from baseline in \( D \) (0.7%), \( V_{\text{max}} \) (−8.2%), and \( Q \) (−7.7%) were not statistically significant in the placebo-treated eyes. In the timolol-treated eyes, there was no significant average change from baseline in \( D \) (0.1%) but a significant average increase in \( V_{\text{max}} \) of 9.6% (\( P < 0.05 \)). The 10% average increase in \( Q \), however, had a \( P \) value of only 0.07.

In comparison with the placebo-treated eyes, \( V_{\text{max}} \) and \( Q \) were both significantly increased by about 18% in the timolol-treated eyes after treatment (\( P < 0.0001 \) and \( P < 0.005 \), respectively; Fig. 2). No significant difference in the percentage changes in \( D \) were observed between the timolol- and placebo-treated eyes.

Although, on average, there was no significant change in \( B P_m \) after treatment, the individual changes in \( B P_m \) correlated significantly with the percentage changes in \( Q \) (\( r = 0.64, P < 0.01 \)) in the timolol-treated eyes. No significant correlation between these quantities was detected in the placebo-treated eyes (\( r = 0.33, P < 0.05 \); Fig. 3).

In addition, significant correlations were also observed between individual changes in PP and changes in either \( V_{\text{max}} \) (\( r = 0.56, P < 0.05 \)) or \( Q \) (\( r = 0.68, P < 0.005 \)) in the timolol-treated eyes; there were no significant associations between these quantities in

**Table 1. Average heart rate, mean brachial artery blood pressure (\( B P_m \)), intraocular pressure, and perfusion pressure before the instillation of placebo and timolol, and then, 2 hr after the instillation of drops on the 15th day of the study**

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>Significance†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (mm Hg)</td>
<td>65 ± 8†</td>
<td>66 ± 10</td>
<td>( P &gt; 0.05 )</td>
</tr>
<tr>
<td>Intraocular pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>76.9 ± 6.4</td>
<td>75.7 ± 6.5</td>
<td>( P &gt; 0.05 )</td>
</tr>
<tr>
<td>Timolol</td>
<td>14.3 ± 2.4</td>
<td>13.7 ± 2.1</td>
<td>( P &gt; 0.05 )</td>
</tr>
<tr>
<td>Perfusion pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>14.3 ± 2.2</td>
<td>10.9 ± 1.5</td>
<td>( P &lt; 0.0001 )</td>
</tr>
<tr>
<td>Timolol</td>
<td>37.0 ± 4.8</td>
<td>36.1 ± 4.9</td>
<td>( P &gt; 0.05 )</td>
</tr>
<tr>
<td></td>
<td>36.9 ± 4.8</td>
<td>38.9 ± 4.2</td>
<td>( P &gt; 0.05 )</td>
</tr>
</tbody>
</table>

* Paired student t-test.
† ± 1 SD.
Table 2. Following treatment, percentage change from baseline in heart rate, mean brachial artery blood pressure (BPm), intraocular pressure (IOP), and perfusion pressure (PP)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Heart rate</th>
<th>BPm</th>
<th>Placebo</th>
<th>Timolol</th>
<th>PP</th>
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<tr>
<td>1</td>
<td>-8.3</td>
<td>2.2</td>
<td>-6.7</td>
<td>-21.4</td>
<td>5.9</td>
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<tr>
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<td>26.3</td>
<td>-7.7</td>
<td>-20.0</td>
<td>-42.9</td>
<td>-2.7</td>
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<tr>
<td>3</td>
<td>5.9</td>
<td>12.9</td>
<td>-6.7</td>
<td>-20.0</td>
<td>23.3</td>
</tr>
<tr>
<td>4</td>
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<td>2.7</td>
<td>0.0</td>
<td>-31.3</td>
<td>6.0</td>
</tr>
<tr>
<td>5</td>
<td>-23.9</td>
<td>-5.0</td>
<td>0.0</td>
<td>-8.3</td>
<td>-7.1</td>
</tr>
<tr>
<td>6</td>
<td>17.4</td>
<td>-10.8</td>
<td>-11.8</td>
<td>-38.9</td>
<td>-10.4</td>
</tr>
<tr>
<td>7</td>
<td>15.4</td>
<td>-18.2</td>
<td>8.3</td>
<td>-16.7</td>
<td>-26.1</td>
</tr>
<tr>
<td>8</td>
<td>-27.5</td>
<td>-18.3</td>
<td>30.0</td>
<td>-9.1</td>
<td>-28.7</td>
</tr>
<tr>
<td>9</td>
<td>14.5</td>
<td>-2.1</td>
<td>-11.1</td>
<td>-27.8</td>
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<td>10</td>
<td>49.0</td>
<td>2.7</td>
<td>0.0</td>
<td>-18.8</td>
<td>3.9</td>
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<tr>
<td>11</td>
<td>0.0</td>
<td>8.9</td>
<td>0.0</td>
<td>-20.0</td>
<td>13.2</td>
</tr>
<tr>
<td>12</td>
<td>-11.7</td>
<td>-14.8</td>
<td>0.0</td>
<td>-28.6</td>
<td>-20.3</td>
</tr>
<tr>
<td>13</td>
<td>-7.7</td>
<td>5.0</td>
<td>-14.3</td>
<td>-46.7</td>
<td>12.0</td>
</tr>
<tr>
<td>14</td>
<td>-11.3</td>
<td>12.4</td>
<td>9.1</td>
<td>-9.1</td>
<td>18.4</td>
</tr>
<tr>
<td>Mean</td>
<td>2.6 ± 20.6†</td>
<td>-2.4 ± 10.3</td>
<td>-3.4 ± 11.7</td>
<td>-22.8* ± 14.2</td>
<td>-1.1 ± 15.9</td>
</tr>
</tbody>
</table>

* Paired student t-test, P < 0.0001.
† One standard deviation.

The results suggest that after 2 weeks of timolol maleate 0.5% treatment there is a significant increase from baseline in average Vmax of about 10%. Although there was also a 10% increase in average Q from baseline, this was not statistically significant. A strong significant difference was present, however, between the changes from baseline in either Vmax (P < 0.0005) or Q (P < 0.01) observed in the placebo- and the timolol-treated eyes.

Discussion

The results suggest that after 2 weeks of timolol maleate 0.5% treatment there is a significant increase from baseline in average Vmax of about 10%. Although there was also a 10% increase in average Q from baseline, this was not statistically significant. A strong significant difference was present, however, between the changes from baseline in either Vmax (P < 0.0005) or Q (P < 0.01) observed in the placebo- and the timolol-treated eyes. In comparison with the placebo-treated eyes, Vmax and Q after treatment were both significantly increased in the timolol-treated eyes by about 18%.

In this study, the eyes that received placebo were described as placebo treated. It is possible, however, that some of these eyes could have been affected by the drug instilled in the fellow eyes. Although a significant decrease in IOP in the placebo-treated eyes was not detected, instillation of timolol in one eye is known to reduce IOP in the contralateral untreated eye. Whether this decrease results from timolol acting through the central nervous system or from a local effect of the drug reaching the fellow eye through the systemic circulation is not known.

In the current study, significant changes in average systemic blood pressure after treatment were also not detected. Previous studies, however, have shown that timolol reduces heart rate and systemic blood pressure. Such a decrease, if present, would certainly affect both eyes. In addition, in a previous work we also reported data suggesting that the instillation of timolol in one eye may produce a small decrease in ophthalmic artery blood pressure in the fellow eye. These potential effects of timolol could be related to the trend toward a decrease in Vmax and Q observed in the placebo-treated eyes.

The main reason for choosing a study design in which timolol and placebo drops were simultaneously given in one measurement session was our interest in the possible effect of the drug on the retinal autoregulation, i.e., the phenomenon by which the retina can maintain an unchanged blood flow despite changes in blood pressure.
Fig. 1. Average percentage changes from baseline in venous diameter (D), maximum erythrocyte velocity (V_{max}), and volumetric blood flow rate (Q) in the placebo- and the timolol-treated eyes. Vertical bars represent the 95% confidence interval for the mean. Bars that do not include the zero line represent average percentage changes that are significantly different from baseline (P < 0.05).

Fig. 2. Comparison of the percentage change from baseline in venous diameter (D), maximum erythrocyte velocity (V_{max}), and volumetric blood flow rate (Q) between the placebo- and the timolol-treated eyes. A statistically significant difference in the percentage changes in V_{max} and Q are present (paired student t-test, P < 0.0005, and P < 0.01, respectively) between the timolol- and the placebo-treated eyes. In comparison to the placebo-treated eyes, V_{max} and Q are significantly increased following treatment in the timolol-treated eyes.
Fig. 3. Percentage change in volumetric blood flow rate (Q) vs. percentage change in mean brachial artery blood pressure (BPm) in the placebo- and the timolol-treated eyes. There is no significant correlation in the placebo-treated eyes (r = -0.33, P > 0.05) and a significant correlation in the timolol-treated eyes (r = 0.64, P < 0.01; Q = 0.12 + 1.25 BPm).

In a previous investigation using a protocol design similar to the one employed here, after a single instillation of the drug, significant positive correlations were found between changes in BPm and changes in either V_max or Q in the timolol-treated eyes. There were no such correlations observed in the placebo-treated eyes. These results showed that changes in V_max and Q followed more passively the BPm changes in the timolol-treated eyes than in the placebo-treated eyes and suggested that timolol may affect the capac-

Fig. 4. Percentage change in maximum erythrocyte velocity (V_max) vs. percentage change in perfusion pressure (PP) in the placebo- and the timolol-treated eyes. There is no significant correlation in the placebo-treated eyes (r = 0.43, P > 0.05) and a significant correlation in the timolol-treated eyes (r = 0.56, P < 0.05; V_max = 0.05 + 0.62 BPm).
Fig. 5. Percentage change in volumetric blood flow rate (Q) vs. percentage change in perfusion pressure (PP) in the placebo- and the timolol-treated eyes. There is no significant correlation in the placebo-treated eyes (r = 0.38, P > 0.05) and a significant correlation in the timolol-treated eyes (r = 0.68, P < 0.005; Q = 0.04 + 0.92 PP).

The results of the current study corroborate our previously published ones by again demonstrating a significant correlation between changes in Q and changes in BPm in the timolol-treated eyes with no significant correlation in the placebo-treated eyes (Fig. 3). There were also significant correlations between changes in either Vmax or Q and changes in PP in timolol-treated eyes and no correlations in the placebo-treated eyes (Figs. 4, 5). These results further support the previous hypothesis that timolol may modulate the retinal autoregulatory response.

Timolol maleate is a nonselective beta-adrenergic receptor-blocking agent widely used in the management of glaucoma. Although the human retinal vessels are not known to have adrenergic innervation, it is possible that an adrenergic influence could be mediated by humoral transmitters activating receptors at the vessel wall. This hypothesis is supported by recent work of Ferrari-Dileo who identified beta-adrenergic receptors in bovine retinal vessels. Timolol maleate could reach these receptors or perhaps other nonadrenergic receptors and produce an effect on the regulatory response of the retinal vasculature.

Animal studies in rabbits show that after the instillation of timolol in one eye the concentration of the drug in the posterior pole of that eye is higher than that present in the plasma or in the posterior pole of the contralateral eye, suggesting that a large amount of drug reaches the posterior pole of the instilled eye by diffusion. It is possible, therefore, that timolol could reach retinal vascular receptors in the timolol-treated eye and produce the different responses to changes in BPm and PP observed in the timolol- and placebo-treated eyes (Figs. 3–5).

Beta-adrenergic blockers are known to produce vasoconstriction in vessels that have a beta-adrenergic vasodilatory influence. In this study, vasoconstriction was not detected. Martin et al. however, reported that a 1-week treatment of timolol maleate 0.5% produced a small vasoconstriction of 4% in the main retinal arteries. Such vasoconstriction would lead to a decrease in Vmax, which is the opposite of what was seen in this study.

Changes in the diameter of the large retinal arterioles, however, may not reflect exactly the changes in the smaller precapillary arterioles that produce the main resistance changes which affect the retinal circulation. Furthermore, our results showing increases in Q in most of the eyes and increases in PP and decreases in Q in all eyes in which PP actually decreased (Fig. 5) strongly suggest that the changes in Q are mainly produced by the changes in PP and less by actual changes in vascular resistance.
Although the hypothesis that timolol may affect autoregulation has been raised, other mechanisms could explain the positive correlations shown in Figures 3-5 in the timolol-treated eyes and the lack of correlations in the placebo-treated eyes. For example, the decreases in IOP, significantly larger in the timolol-treated eyes than in the placebo-treated eyes, could have a larger effect on V_{max} and Q in the timolol- than in the placebo-treated eyes. This explanation does not seem plausible, however, since no correlations between IOP changes and changes in either V_{max} or Q in the timolol- or placebo-treated eyes were detected.

In conclusion, the mechanism by which timolol maleate affects the retinal circulation is probably related to the increase in PP produced by the drug and a possible influence on the autoregulatory capacity of the retinal circulation. Our results suggest that the effect of timolol on the retinal circulation is maintained after 14 days of treatment since the average changes in V_{max} and Q were similar to those previously reported after one instillation of the drug in normal eyes and eyes with ocular hypertension. Whether this effect may be beneficial in eyes with retinal diseases in which the vascular perfusion is decreased remains to be investigated.

**Key words:** laser Doppler velocimetry, ocular hypertension, timolol maleate, volumetric blood flow rate, erythrocyte velocity, autoregulation, human retina

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**References**