Effect of Timolol Maleate on the Retinal Circulation of Human Eyes with Ocular Hypertension

Juan E. Grunwald

We studied the effect of topical timolol maleate 0.5% on the retinal circulation of eyes with ocular hypertension using laser Doppler velocimetry and monochromatic fundus photography. Patients with ocular hypertension had normal eye examinations and had documented elevated intraocular pressures of 23 mmHg or higher on two or more separate occasions. In a double-masked randomized design, one eye of each subject received timolol maleate 0.5% and the fellow eye received placebo. Vessel diameter, maximum velocity of red blood cells, and volumetric blood flow rate were determined in a major retinal vein of each eye just prior to the instillation of drops, and then 2 hr later. In comparison to the baseline value, there was an increase of 12.0% in average red blood cell velocity (P < 0.005, statistically significant) and 8.4% in volumetric blood flow rate in the timolol-treated eyes (P < 0.05, statistically significant). No significant changes in these quantities were observed in the placebo-treated eyes. Also, no significant change in venous diameter was detected in the placebo- and the timolol-treated eyes. In comparison to the baseline, a significantly larger increase in red blood cell velocity was observed in the timolol-treated eyes than in the placebo-treated eyes (P < 0.05). The difference between the increase in blood flow observed in the timolol-treated eyes and the placebo-treated eyes achieved a probability value of \( P = 0.058 \). The increase in blood flow observed in the timolol-treated eyes may be related to the increase in perfusion pressure produced by this drug. Invest Ophthalmol Vis Sci 31:521-526, 1990

Materials and Methods

This study was carried out in 14 healthy volunteers aged 25-72 yr (mean 53 ± 14 yr, ±1 SD) with no history of systemic decrease or intraocular disease other than increased intraocular pressure (IOP). Corrected visual acuities were 6/7.5 or better. Slit-lamp and funduscopic examinations were normal. Gonioscopic examination revealed a wide open angle in all of the eyes. Subjects had documented IOPs of 23 mmHg or higher on at least two occasions prior to our blood flow measurements. All subjects had no evidence of visual field defects on Octopus Perimetry (Program 32) and had optic nerve heads with a cup-to-disc ratio of 0.5 or less. None of the patients was taking topical or systemic medication at the time of the study. Informed consent was obtained from each subject.

After pupillary dilatation with tropicamide 1%, a Polaroid color fundus photograph of the disc was taken. Bidirectional laser Doppler measurements of red blood cell (RBC) velocity in a main superior or inferior temporal retinal vein were obtained in both eyes. BLDV determinations were performed 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 previously. Therefore, we provide only a summary here.

The maximum or centerline velocity of the RBCs (Vmax) was determined according to the relation: Vmax = KΔf, where K is a constant related to the scattering geometry and the wavelength of the laser light. Δf = fmax - f2max, where fmax and f2max are the cut-off frequencies of the Doppler shift power spectra recorded simultaneously in two different directions of the scattered light. Approximately 20 pairs of power spectra were used to calculate an average Δf(Δf) from which Vmax was obtained.

Immediately after the BLDV recordings, fundus photographs were taken in monochromatic light at 570 nm using a Zeiss (Carl Zeiss, Oberkochen, West Germany) fundus camera and Kodak (Eastman Kodak, Rochester, NY) Plus-X pan film. 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 photographs and BLDV determinations were performed in a darkened room and in a sitting position.

Retinal volumetric blood flow rate, Q, was calculated from the relation: Q = Vmean × πD2/4, where Vmean represents the mean velocity of whole blood. We assumed that Vmean = C Vmax, with C as a constant that is the same for all vessels measured. In this study, we adopted a value of C = 1/1.6, based on the work of Damon and Duling,9 who studied the relationship between Vmax and Vmean by using glass tubes.

After measurements of baseline BLDV and MFP were made, the heart rate was determined, and systolic and diastolic blood pressures were measured by sphygmomanometry. Two drops of topical proparacaine HCl 0.5% were instilled in each eye, and 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, and the fellow eye received one drop of placebo, consisting of the vehicle of timolol ophthalmic solution. Two hours later, this experimental procedure was repeated. Subjects were asked to refrain from eating or drinking during this 2-hr period.

Mean brachial artery blood pressure, BPm, was calculated according to the formula BPm = BPd + 1/3 (BP S - BP d), where BP S and BP d are the brachial artery systolic and diastolic pressures, respectively. Perfusion pressure, PP, was calculated as PP = 2/3 BPm - IOP.

All measurements of vessel diameter were performed by one trained examiner, and all Vmax determinations were done by another trained examiner. Each individual was masked with regard to: 1) the results of the other examiner, 2) whether measurements were obtained at baseline or after treatment, and 3) which eye had received timolol.

Statistical evaluation of the data was performed with the 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.

Results

Average blood pressure, IOP, perfusion pressure, and heart rate before and after the instillation of the drops are shown in Table 1. After treatment, there was no significant change in heart rate or mean brachial artery blood pressure. The average IOP decreased significantly, by 22% in the eyes that received placebo (paired student t-test, P < 0.0005), and by 38% in the eyes that received timolol (P < 0.0001). The average perfusion pressure, on the other hand, showed a nonsignificant increase of 8% in the placebo-treated eyes (P > 0.05) and a significant increase of 21% in the timolol-treated eyes (P < 0.0001).

The average values of D, Vmax, and Q before and after the instillation of placebo and timolol drops are summarized in Table 2. Figure 1 shows the average percentage change from baseline in D, Vmax, and Q for the placebo- and timolol-treated eyes. After treatment, the average change from baseline in D, Vmax, or Q was not statistically significant in the placebo-treated eyes. In the timolol-treated eyes, there was no significant difference from baseline in D, but there were significant average increases of 12.0% in Vmax (P < 0.0005) and of 8.4% in Q (P < 0.05). In comparison to the baseline value, the percentage increases in Vmax were significant average increases of 12.0% in Vmax (P < 0.05) and 38% in the eyes that received timolol

Table 1. Average heart rate, BPm, IOP, and PP before and after the instillation of placebo or timolol

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>Significance*(P)</th>
</tr>
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<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>72 ± 7**</td>
<td>71 ± 8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BPm (mmHg)</td>
<td>97.0 ± 8.7</td>
<td>94.9 ± 8.6</td>
<td>&gt;0.05</td>
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<tr>
<td>IOP (mmHg)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Placebo</td>
<td>24.4 ± 2.4</td>
<td>18.9 ± 5.1</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Timolol</td>
<td>24.8 ± 2.7</td>
<td>15.5 ± 2.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>40.5 ± 5.4</td>
<td>43.6 ± 9.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Timolol</td>
<td>40.1 ± 6.2</td>
<td>47.9 ± 5.5</td>
<td>&lt;0.0001</td>
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* Paired student t-test.
** ± 1 SD.
Table 2. Average D, $V_{\text{max}}$, and Q before and after the instillation of placebo or timolol

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>Significance* (P)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D$ ($\mu m$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>$157 \pm 26^*$</td>
<td>$157 \pm 28$</td>
<td>$&gt;0.05$</td>
</tr>
<tr>
<td>Timolol</td>
<td>$154 \pm 27$</td>
<td>$153 \pm 26$</td>
<td>$&gt;0.05$</td>
</tr>
<tr>
<td>$V_{\text{max}}$ (cm/sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>$1.69 \pm 0.38$</td>
<td>$1.68 \pm 0.36$</td>
<td>$&gt;0.05$</td>
</tr>
<tr>
<td>Timolol</td>
<td>$1.71 \pm 0.44$</td>
<td>$1.93 \pm 0.55$</td>
<td>$&lt;0.005$</td>
</tr>
<tr>
<td>Q ($\mu l/min$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>$12.8 \pm 6.2$</td>
<td>$12.7 \pm 6.4$</td>
<td>$&gt;0.05$</td>
</tr>
<tr>
<td>Timolol</td>
<td>$12.8 \pm 6.9$</td>
<td>$13.9 \pm 7.8$</td>
<td>$&lt;0.05$</td>
</tr>
</tbody>
</table>

* Paired student t-test.
** ± 1 SD.

were significantly greater in the timolol-treated eyes than in the placebo-treated eyes ($P < 0.05$). The difference between the percentage increases in Q in the timolol-treated eyes and the placebo-treated eyes achieved a probability value of $P = 0.058$.

After the instillation of the drops, significant correlations were found between the individual percentage changes in PP and the percentage changes in D ($r = -0.76$, $P < 0.005$; Fig. 2); between the percentage changes in $BP_m$ and the percentage changes in D ($r = -0.57$, $P < 0.04$; Fig. 3); and between the percentage changes in IOP and the percentage changes in D ($r = 0.78$, $P < 0.001$; Fig. 4) or Q ($r = 0.71$, $P < 0.005$; Fig. 5) in the placebo-treated eyes. Except for those correlations just mentioned in the placebo eyes, no other significant correlations were detected between individual percentage changes in either IOP, PP, or $BP_m$ and individual changes in D, $V_{\text{max}}$, or Q in the placebo- or timolol-treated eyes.

**Discussion**

This study demonstrated a significant average increase of 12% in the maximum velocity of red blood cells, $V_{\text{max}}$, 2 hr after the instillation of timolol ($P < 0.005$). It demonstrated also a significant average increase in volumetric blood flow rate, Q, in the timolol-treated eyes ($P < 0.05$). This increase, however, was of borderline significance ($P = 0.058$) when the changes in Q obtained in the timolol-treated eyes were compared with those obtained in the placebo-treated fellow eyes. These results are similar to those of a previous study in normal eyes, which showed an average increase of 11% in $V_{\text{max}}$ and of 13.2% in Q in the timolol-treated eyes.6

The finding of a significant decrease in IOP in both the timolol- and the placebo-treated eyes is in agreement with previous reports.2,4,6 Whether this decrease resulted from timolol acting by way of the central nervous system, or from a local effect of the drug...
reaching the fellow eye through the systemic circulation, \(10,11\) is not known. We emphasize, therefore, that although we described eyes as placebo-treated eyes, these eyes probably are affected by the timolol delivered in the fellow eye.

We have reported previously \(6\) that in the timolol-treated eyes there is a close similarity between the average percentage changes in perfusion pressure and the average percentage changes in \(Q\), and a positive significant correlation between the individual changes in these quantities. These findings suggested that the increases in \(Q\) are produced by the increases in perfusion pressure. We therefore hypothesized that since in ocular hypertensive eyes timolol causes a larger IOP drop and a larger increase in perfusion pressure than in normal eyes, the expected effect of timolol on \(Q\) would be larger than that found in normal eyes. The results of the current study, however, do not support this hypothesis, since the changes found in \(Q\) were not greater than those found previously in normals. A possible explanation for the absence of a greater response is that our ocular hypertensive patients were older than our previously studied normals.

Our current results in the placebo-treated ocular hypertensive eyes show several noteworthy correlations (Figs. 2–5) that suggest an active autoregulatory response to the changes in perfusion pressure. The eyes that had decreases in perfusion pressure or mean brachial artery blood pressure tended to show vasodilation of the retinal veins, whereas those that had increases in perfusion pressure or mean brachial artery blood pressure showed vasoconstriction (Figs. 2, 3). A positive correlation was seen also in the placebo-treated eyes between changes in IOP (Fig. 4) and changes in \(D\). In other words, eyes with greater IOP decreases showed greater decreases in \(D\) (Fig. 4).

The position of the regression line in Figure 4 suggests that no changes in \(D\) occurred with decreases in IOP of about 20%. A partial explanation for this phenomenon may be found in our previous work, performed in normal eyes, which suggested that there
may be a small decrease (average approximately 3 mmHg, \( P = 0.052 \)) in mean ophthalmic artery blood pressure in the placebo-treated eyes under experimental conditions similar to those of our current work.\(^{12}\) This phenomenon may be related to the known systemic effect of the timolol drop given in the fellow eye, which produces a decrease in systemic blood pressure.\(^{13}\) Concurrent small decreases in IOP and ophthalmic artery blood pressure would leave the perfusion pressure largely unaffected, and therefore, no regulatory change in D would be needed for such a small decrease in IOP.

Figure 5 shows that in the placebo-treated eyes there is a trend towards a decrease in Q in eyes with greater declines in IOP. The lack of significant correlation between IOP changes and changes in \( V_{\text{max}} \) suggests that these changes in Q were due mainly to changes in D.

Autoregulation is defined usually as the phenomenon by which the retina can maintain constant blood flow in response to changes in perfusion pressure. The results of Figure 5 show, however, that in eyes with greater decreases in IOP (greater increases in perfusion pressure), there was a tendency towards decreases in Q instead of the unchanged Q that would be expected from an autoregulatory response.

The purpose of autoregulation is to maintain an adequate supply of and removal of metabolites in the retina despite changes in perfusion pressure. It is possible that in eyes with ocular hypertension, greater decreases in IOP produce increases in choroidal blood flow and increased oxygen delivery to the retina,\(^{14}\) which in turn could lead to the retinal vasoconstriction and the decrease in Q suggested by the results of Figure 5.

Previous investigators who used the radioactive microsphere technique in the normal rabbit eye did not detect any significant effect of topical timolol on the retinal circulation.\(^{15,16}\) The discrepancy between these studies and the current study could be due to species differences, since the circulation of the human and rabbit retinae are very different. In addition, the limited accuracy and sensitivity of the microsphere technique, limitations which are due to the small number of microspheres that accumulate in the retinal tissue,\(^{17}\) may have precluded the detection of small changes in retinal volumetric blood flow rate. It is noteworthy, however, that another study of the effect of timolol on the choroidal circulation of the cat also has shown an increase in blood flow.\(^{18}\)

Richard and Weber\(^{19}\) have suggested that timolol maleate 0.5% may produce an increase in human retinal circulation time, as detected by fluorescein videangiography, and a decrease in the number of entoptic particles observed within 30 sec in a specific area of the blue-field entoptic phenomenon. In both methods used by Richard and Weber, a subjective bias on the part of the investigators or the experimental subjects could affect the results. Therefore, the lack of a placebo and appropriate masking procedures weaken the conclusions of their work, suggesting that retinal blood flow may be decreased following timolol instillation. Furthermore, the increase in arm-to-retina circulation time found by Richard and Weber\(^{19}\) represents a systemic effect of the drug, resulting perhaps from a decrease in systemic blood pressure or cardiac rate.\(^{13}\) This systemic effect could result from the instillation of the drug three times, and would tend to reduce retinal blood flow.

Another significant difference between the current study and that of Richard and Weber\(^{19}\) is the timing of the measurements. Richard and Weber obtained their measurements 30 min after the instillation of the drug, whereas we obtained measurements at 120 min. Since the full effect of timolol on IOP and perfusion pressure is reached only after about 120 min,\(^{20}\) some of the differences in the results of the two studies may be explained by this timing difference.

In summary, our study shows that in eyes with ocular hypertension, topical timolol maleate produces a small increase in blood velocity, probably accompanied by an increase in blood flow. Whether topical timolol has a similar effect in ocular hypertensive eyes in which the retinal circulation may be compromised by a vascular occlusion needs to be studied.

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

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