Effect of thymoxamine on aqueous humor formation in the normal human eye as measured by fluorophotometry

David A. Lee, Richard F. Brubaker, and Shigetoshi Nagataki

A double-blind, randomized, placebo-controlled study of the effect of 0.5% topical thymoxamine hydrochloride, an alpha-adrenergic antagonist, on the rate of aqueous humor formation in the eyes of 25 normal human subjects was performed with fluorophotometry. The effect of thymoxamine on intraocular pressure and anterior chamber volume was also studied. Four of the 25 subjects were used to study the effect of thymoxamine on the permeability to fluorescein of the blood-aqueous barrier. Pupillography was used to confirm that alpha blockade was present in the iris. Thymoxamine-treated eyes had 12% greater aqueous humor flow than placebo-treated eyes, but this difference was not statistically significant. No statistically significant difference was found in the rate of aqueous humor formation, intraocular pressure, anterior chamber volume, or permeability of the blood-aqueous barrier between thymoxamine-treated and placebo-treated eyes. The lack of an effect on the aqueous system could be interpreted as being a result of too low a concentration of thymoxamine in the ciliary body, lack of blockable receptors in the ciliary body, lack of physiologic tone in a blockable receptor system, or lack of a role of such a receptor system in the formation of aqueous humor. (INVEST OPHTHALMOL VIS SCI 21:805-811, 1981.)

Key words: aqueous humor dynamics, fluorophotometry, thymoxamine, alpha antagonist, intraocular pressure, anterior chamber volume, normal human eye

There is a relative scarcity of information in the literature about the role of alpha receptors in controlling the rate of aqueous humor formation in the human eye. It is unknown whether alpha-stimulating or alpha-blocking agents may be useful in certain clinical situations where a change in the rate of aqueous humor formation is medically advantageous.

Thymoxamine hydrochloride is a selective alpha-adrenergic blocking agent that acts by competitive antagonism. The alpha selectivity of thymoxamine has been well documented. This drug was first used in the eye in 1955 and has been used in many subsequent ocular experiments.

The use of thymoxamine as an alpha-adrenergic blocker in the eye has been reviewed extensively by Wand and Grant. The primary use of this drug in the eye has been to produce miosis without accommodation for both diagnostic and therapeutic reasons. Thymoxamine produces miosis without an accompanying shallowing of the anterior chamber. Rutkowski et al. have shown that this effect of thymoxamine is useful for the treatment of acute angle-closure glaucoma. Wand and Grant have used thymoxamine as a diagnostic test for differentiating open-angle glaucoma with narrow angles from angle-closure

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between the posterior surface of the iris and glaucoma. Campbell\textsuperscript{10} has proposed that thymoxamine might be useful in pigmentary glaucoma by reducing the mechanical contact between the posterior surface of the iris and the anterior surface of the zonular fibers.

There have also been studies on the effect of thymoxamine on intraocular pressure and tonographic facility of outflow. In one study, 0.5% thymoxamine was found to reduce intraocular pressure in normal subjects.\textsuperscript{11} In another study, the 1% solution was found to have no effect on intraocular pressure in seven patients with open-angle glaucoma, but the facility of outflow was found to be reduced 1 week after continued use.\textsuperscript{12} More recent studies have demonstrated that 0.5% thymoxamine does not influence intraocular pressure in normal subjects.\textsuperscript{7, 13} or in patients with open-angle glaucoma.\textsuperscript{13} Likewise, no effect on facility of outflow was found in patients with open-angle glaucoma or in normals.\textsuperscript{13}

It would appear therefore that alpha blocking agents either have no effect on aqueous humor dynamics or have complementary effects on inflow and outflow that cannot be detected by tonometric techniques. Since thymoxamine is a potent alpha-adrenergic antagonist that has been shown to be safe to administer as a topical solution to the human eye, it is a logical drug to use to help clarify the role of alpha-adrenergic receptors in aqueous humor dynamics. Any effect that might be present, as regards aqueous humor formation, could be determined in humans by using dye dilution techniques with fluorescein.\textsuperscript{14-16}

### Table I

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<th>Sex</th>
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<th>Final pressure (mm Hg)</th>
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Mean 28.34                                    12.6                      11.3                      11.4                      194.9                      193.4                      1.45                      2.48
S.D. 4.58                                      2.5                      2.2                      2.0                      33.5                      31.7                      0.38                      0.62
S.E. 0.92                                      0.5                      0.4                      0.4                      6.7                       6.3                       0.075                     0.12

AC = Anterior chamber.
Totals—Sex: 12 M, 13 F; eye color: 18 L, 7 D; thymoxamine: 9 R, 16 L; placebo: 16 R, 9 L.
Materials and methods

Twenty-five normal human subjects, 12 males and 13 females, were studied. An eye examination of each subject was carried out, consisting of best corrected visual acuity, external examination, slit-lamp examination, fundus examination, and tonometry. All subjects met the admission criteria that both eyes be normal (except for ametropia) and that the intraocular pressure of the two eyes differ by 3 mm Hg or less.

Thymoxamine hydrochloride ophthalmic solution (0.5%) IND 17,002 was used along with an identical-appearing placebo vehicle solution containing all the same ingredients except thymoxamine. The drug-placebo bottle pairs were randomized by a hospital pharmacy to the right-left eye pairs of the subjects. The containers were labeled as follows: Subject 1, right eye; Subject 1, left eye; Subject 2, right eye; Subject 2, left eye; etc. Neither the examiners nor the subjects knew which eye had received thymoxamine until the study was completed. The code was not broken until the data of all the subjects had been calculated. In determining the effect of the drug, the treated eye of the subject was compared with the fellow placebo eye. The concentration of thymoxamine hydrochloride was measured immediately after formulation and after the conclusion of the entire experiment to assure that the correct percentage was being used and that the drug did not undergo chemical degradation during storage.

On the morning of the study, a baseline Lowenstein-Lowenstein pupillogram was taken of both eyes, with light and sound stimuli. Then the volume of the anterior chamber of both eyes was measured by the photogrammetric method of Johnson et al. The color of the irides was noted. After photography, 1 drop of drug or placebo solution was instilled into each eye. Thirty minutes later, background mass measurements were taken with the flash fluorophotometer described by Brubaker and Coakes. Then both eyes of the sub-

\[
\begin{array}{cccccccc}
\text{Eye (right/ left)} & \text{Initial pressure (mm Hg)} & \text{Pressure 30 min after Rx (mm Hg)} & \text{Final pressure (mm Hg)} & \text{Initial AC volume (\(\mu\ell\))} & \text{Final AC volume (\(\mu\ell\))} & K_+ (\text{mm Hg}^{-1} \times 10^2) & \text{Aqueous flow (\(\mu\ell\) min\(^{-1}\))} \\
L & 17 & 12 & 16 & 211 & 201 & 1.51 & 2.73 & -1.5 \\
L & 14 & 12 & 13 & 273 & 233 & 1.07 & 2.34 & +6.7 \\
L & 14 & 10 & 11 & 139 & 159 & 1.41 & 2.01 & +42.3 \\
R & 14 & 9 & 12 & 179 & 170 & 1.55 & 2.38 & -25.6 \\
R & 10 & 10 & 11 & 164 & 164 & 1.37 & 2.03 & +70.9 \\
R & 16 & 12 & 10 & 200 & 190 & 0.65 & 1.11 & +137.8 \\
R & 10 & 14 & 12 & 215 & 205 & 1.19 & 2.20 & -4.1 \\
R & 12 & 9 & 10 & 196 & 195 & 1.32 & 3.19 & +4.4 \\
L & 12 & 9 & 8 & 204 & 195 & 1.43 & 2.51 & -12.8 \\
L & 14 & 14 & 12 & 207 & 210 & 1.54 & 2.91 & -4.5 \\
L & 10 & 9 & 8 & 183 & 173 & 1.74 & 2.71 & -10.3 \\
R & 14 & 11 & 13 & 195 & 184 & 1.41 & 2.33 & -3.9 \\
L & 16 & 10 & 10 & 249 & 274 & 1.25 & 2.09 & +4.9 \\
L & 10 & 10 & 11 & 187 & 189 & 1.12 & 1.90 & +75.8 \\
R & 10 & 8 & 12 & 166 & 186 & 1.09 & 1.82 & -8.8 \\
R & 10 & 10 & 9 & 206 & 196 & 1.44 & 2.54 & -9.5 \\
R & 10 & 14 & 13 & 266 & 282 & 1.41 & 3.58 & +5.0 \\
R & 12 & 14 & 11 & 195 & 192 & 1.24 & 2.15 & -7.0 \\
R & 14 & 12 & 13 & 232 & 219 & 1.10 & 2.17 & -29.5 \\
R & 9 & 13 & 14 & 227 & 229 & 0.92 & 1.90 & +40.0 \\
R & 10 & 11 & 12 & 186 & 210 & 0.78 & 1.48 & +33.1 \\
R & 9 & 10 & 10 & 246 & 250 & 1.61 & 3.61 & -57.1 \\
R & 16 & 16 & 16 & 111 & 120 & 2.08 & 2.46 & -18.3 \\
L & 11 & 11 & 10 & 138 & 150 & 1.26 & 1.71 & +39.2 \\
R & 16 & 16 & 14 & 206 & 195 & 1.19 & 2.08 & +2.9 \\
2.4 & 11.4 & 11.6 & 199.2 & 198.8 & 1.34 & 2.35 & +11.6 \\
2.6 & 2.2 & 2.1 & 38.5 & 36.2 & 0.34 & 0.61 & 39.9 \\
0.5 & 0.4 & 0.4 & 7.7 & 7.2 & 0.068 & 0.12 & 0.0
\end{array}
\]
ject were anesthetized with 0.5% proparacaine, and fluorescein iontophoresis was carried out in the central 4 mm of the cornea with an electrode of 10% fluorescein in 2% agar. A current of 200 μA was used for 7 sec, depositing 0.3 to 0.9 μg of fluorescein beneath the epithelium. The conjunctival sac was irrigated with balanced salt solution to remove any surface fluorescein. Then intraocular pressure was measured with the Goldmann applanation tonometer. Flourescin concentration and mass were measured in the cornea and the anterior chamber at 0.5, 2, and 7 hr after iontophoresis with the fluorescein beneath the epithelium. Then the fasting subject drank 1 gm of fluorescein mixed in 8 oz of orange juice. Fluorescein mass and concentration were measured in the anterior chamber every 30 min for 4 hr.

Two-sided tests to determine statistical significance were used, accepting a probability level of p \leq 0.05 as significant. For paired data, the t test for paired samples and the paired Wilcoxon tests were used. For unpaired data, the t test for sample means and the Mann-Whitney Test (corrected for ties) were used.

**Results**

Table I outlines the results of each subject and illustrates the mean, standard deviation, and standard error of each measured and calculated parameter for the entire group. Table II outlines the means, standard deviations, and standard errors of thymoxamine and placebo-treated eyes along with their subgroups, including right/left, male/female,
and light/dark irides. Thymoxamine-treated eyes had 12% greater aqueous humor flow than placebo-treated eyes, but this difference was not statistically significant. No statistically significant differences were seen between thymoxamine- and placebo-treated eyes or among their subgroups in any parameter.

There was no statistically significant difference in the total mass and anterior chamber concentration of fluorescein (after oral administration) between thymoxamine- and placebo-treated eyes as shown in Fig. 1.

No adverse side effects from thymoxamine were reported by any of the subjects in this study, except a sensation of burning, reported by a few subjects, just after the drug was instilled.

Discussion

The results of this study indicate that thymoxamine does not significantly change aqueous humor formation, intraocular pressure, or anterior chamber volume in the normal human eye.

Our previous experiments with acute topical doses of beta-adrenergic blockers in the normal human eye would indicate that these drugs reduce the rate of formation of aqueous humor.21-24 In this study, the observed effect of the alpha-adrenergic blocking agent was to increase aqueous flow slightly, but the increase could have been due to chance. Moreover, the effect was so small as to lead us to the conclusion that the drug has no biologically significant effect on aqueous formation.

It cannot be determined from our data whether we achieved high enough drug levels in the eye to affect the ciliary body or not. However, we have documented, using pupillography, that this dosage and lower dosages of thymoxamine produce miosis, presumably because of alpha blockage. The lack of an effect on aqueous formation could have been due to low concentration in the ciliary body or to lack of an active receptor-response system important in the biological chain of events that leads to aqueous formation, which could be blocked by this drug. We favor the latter interpretation of the data.

It is noteworthy that the level of fluorescein that appeared in the anterior chamber of the treated and untreated eye was the same after oral fluorescein. We interpret this
finding as indicating that the drug has no appreciable effect on the permeability of the blood-aqueous barrier to fluorescein and, in particular, on the permeability of the iris to fluorescein. Thus the lack of change in $K_a$ can be interpreted as being due to a lack of effect on either of its components $K_d$ (diffusion) and $K_f$ (flow) rather than being due to equal and opposite effects on these two mechanisms of fluorescein loss.

REFERENCES


