Increased sensitivity to theophylline associated with primary open-angle glaucoma

Harry A. Zink, Paul F. Palmberg, and John F. Bigger

It has previously been reported that lymphocytes from patients with primary open-angle glaucoma and glucocorticoid-induced ocular hypertension demonstrate an increased sensitivity to glucocorticoids in vitro. Using the same in vitro assay we have studied the response of the lymphocyte to theophylline. When compared to nonglaucomatous control subjects, the lymphocytes from patients with primary open-angle glaucoma and glucocorticoid-induced ocular hypertension demonstrated an increased sensitivity to theophylline (p < 0.001). The concentration of theophylline necessary to inhibit by 50 per cent the uptake of tritiated thymidine into DNA correlated closely with values obtained for prednisolone-21-phosphate (r = +0.748). The possibility that the increased glucocorticoid sensitivity present in lymphocytes may be mediated by cyclic AMP is discussed.

Key words: open-angle glaucoma, intraocular pressure, lymphocyte transformation, theophylline, phytohemagglutinin, glucocorticoids, cyclic AMP.

Primary open-angle glaucoma is an ocular disease manifested by increased intraocular pressure, visual field loss, and gonioscopically open anterior chamber angles. While frequently observed to occur in a familial pattern, its exact mode of inheritance remains controversial.1-3

There is evidence to suggest that at least one factor in the etiology of open-angle glaucoma is a recessively inherited sensitivity to glucocorticoids.1,4,5 For example, it has been demonstrated that glucocorticoids administered topically to the human eye will induce a marked elevation of the intraocular pressure in certain individuals. Such a response occurs in 92 per cent of patients with primary open-angle glaucoma as contrasted with only 4 per cent of a normal, nonglaucomatous population.1,5

Recent data suggest that increased sensitivity to glucocorticoids in open-angle glaucoma is not confined to the eye, but can also be detected systemically. Bigger, Palmberg, and Becker6 using glucocorticoids to inhibit phytohemagglutinin-induced lymphocyte transformation, demonstrated an increased responsiveness to glucocorticoids both in individuals with open-angle glau-
coma and glucocorticoid-induced ocular hypertension.

It is not known by what mechanism glucocorticoids function in either increasing intraocular pressure or in inhibiting lymphocyte transformation. The purpose of the present study was to investigate the possibility that the adenyl cyclase system might be involved in the previously demonstrated differential sensitivity to glucocorticoids in the human lymphocyte. We have compared the effect of theophylline, an inhibitor of cyclic AMP phosphodiesterase, with the effect of prednisolone-21-phosphate on lymphocyte transformation in individuals whose ocular response to glucocorticoids had previously been determined.

Method

Twenty-seven patients, selected from the clinical service of the Department of Ophthalmology, and from the Glaucoma Research Center at Washington University, were included in the present study. There were seven patients with primary open-angle glaucoma (OAG) and characteristic visual field loss, ten individuals who did not respond (NN) with an intraocular pressure elevation to topical dexamethasone testing, and ten individuals who were classified as high-pressure responders (GG) to topical dexamethasone but did not have visual field loss. Age distribution was similar in the three groups, and individuals with known neoplastic disease, diabetes mellitus, and those on estrogen therapy were excluded from the study.

The culture medium used was a modification of TC-199 with HEPES buffer, containing 15 per cent non-heat-inactivated fetal calf serum, prepared as described previously. The method of preparation of lymphocyte suspensions, phytohemagglutinin stimulation, harvesting, and scintillation counting has also been reported previously. One-tenth milliliter of 2.0 μM tritiated thymidine (specific activity 20 Ci per millimole) was added to each culture tube after 48 hours of incubation and 16 hours prior to harvesting.

In the present study, for each patient we prepared two unstimulated blanks, six phytohemagglutinin-P (PHA-P)-stimulated control samples, quadruplicates of each of four concentrations of prednisolone-21-phosphate with PHA-P, and quadruplicates of each of four concentrations of theophylline with PHA-P. In each case the prednisolone-21-phosphate or theophylline was added to the cell suspension and allowed to incubate for one hour prior to the addition of PHA-P.

For each patient two dose-response curves for inhibition of tritiated thymidine incorporation into phytohemagglutinin-stimulated lymphocytes were obtained: one for inhibition by prednisolone-21-phosphate, and the second for inhibition by theophylline. Counts per minute (CPM) incorporation corrected for the nonstimulated blank was plotted vs. log concentration of the drug. Regression analysis for data was performed. Half inhibition concentrations (I50) for each patient and each drug were determined by the intersection of the dose-response regression line with a horizontal line at 50 per cent of the mean PHA-P-stimulated control incorporation.

In an additional experiment a series of dose-response curves for prednisolone-21-phosphate inhibition were determined for one patient in the presence of three different concentrations of theophylline.

Results

Fig. 1 shows a dose-response curve for the inhibition by theophylline of tritiated thymidine uptake into DNA in PHA-P-stimulated lymphocytes. This curve was obtained from a patient with primary open-angle glaucoma. The 50 per cent inhibitory concentration (I50) of theophylline was 21 × 10^-5 molar.

A plot of the I50 values for theophylline inhibition for each of the 27 patients by ocular classification is shown in Fig. 2. Nine of the ten glucocorticoid nonresponders (NN) had I50 values greater than 34 × 10^-5 M, while all of the open-angle glaucoma (OAG) and glucocorticoid ocular high responder (GG) individuals had I50 values below this value.

The mean I50 and standard error of the mean (SEM) for each of the three groups, and statistical comparisons of the OAG and GG groups to the NN group are shown in Table I. The mean I50 for the OAG and GG groups, respectively, is lower than for the NN group, a difference which is statistically highly significant (p < 0.001).* The I50 values for the OAG and GG groups do not differ significantly (p > 0.2).

*Applanation ocular pressure < 20 mm. Hg after six weeks of topical application of 0.1 per cent dexamethasone drops four times a day.
†Applanation ocular pressure > 31 mm. Hg during six weeks of topical testing.

*Student two-tailed t test.
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Fig. 1. Dose-response curve for theophylline inhibition of lymphocyte transformation, using lymphocytes from a patient with primary open-angle glaucoma. The abscissa represents the theophylline molar concentration plotted on a logarithmic scale. The ordinate represents counts per minute (CPM) \( \times 10^4 \) for tritiated thymidine incorporation. Phytohemagglutinin-stimulated control samples with no theophylline are represented by 0 on the abscissa. Data are plotted as mean CPM ± one standard deviation. The horizontal dashed line represents 50 per cent of control thymidine uptake.

A plot of the I_{50} for theophylline inhibition vs. the I_{50} for prednisolone-21-phosphate inhibition for each patient is shown in Fig. 3. Linear regression analysis revealed a correlation coefficient (r) of 0.748. This demonstrates a highly significant statistical correlation between the I_{50} values for the two compounds (p < 0.001). There was no significant correlation between the theophylline I_{50} values and the patient's plasma cortisol value, age, race, sex, or with the amount of transformation (p. > 0.2).

Fig. 4 shows three dose-response curves for prednisolone-21-phosphate inhibition of lymphocyte transformation in the presence of different concentrations of theophylline. The inhibitory effect on transformation by the two drugs appears to be additive. The three curves have essentially the same slope (0.490, 0.485, and 0.484) when calculated as per cent decrease in CPM per log unit change in concentration of prednisolone-21-phosphate.

Discussion

Cyclic AMP has been implicated in multiple intracellular functions acting as a "second messenger" in metabolic regulation by hormones. It is generated from ATP by the enzyme adenyl cyclase and is hydrolyzed to the inactive 5' AMP by adenosine monophosphate phosphodiesterase. The concentration of cyclic AMP can be increased by compounds which either
stimulate adenyl cyclase or inhibit phosphodiesterase.\textsuperscript{1}

Cyclic AMP, as well as compounds which increase the intracellular concentration of cyclic AMP, will inhibit phytohemagglutinin-induced lymphocyte transformation.\textsuperscript{5-10} In the present study we used theophylline, a methyl xanthine compound which competitively inhibits phosphodiesterase\textsuperscript{11} thus increasing cyclic AMP, to compare its inhibitory effect on lymphocyte transformation with that previously reported for prednisolone-21-phosphate. The demonstration of a differential responsiveness to theophylline with a greater sensitivity in lymphocytes from patients with open-angle glaucoma and glucocorticoid-induced ocular hypertension parallels our observations with prednisolone-21-phosphate.\textsuperscript{6} The similarity of the response ob-
**Table I.** Theophylline concentration to inhibit lymphocyte transformation by 50 per cent ($I_{50}$)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Patients</th>
<th>Mean $I_{50}$</th>
<th>SEM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>10</td>
<td>39.8</td>
<td>1.6</td>
<td>—</td>
</tr>
<tr>
<td>GC</td>
<td>10</td>
<td>26.4</td>
<td>1.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>OAG</td>
<td>7</td>
<td>24.3</td>
<td>1.8</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

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Obtained to the two different compounds, plus the demonstrated additive effect of theophylline and prednisolone-21-phosphate, suggests that the cyclic AMP system may mediate the glucocorticoid action in human lymphocytes. Additional support for this concept is the demonstration that glucocorticoids decrease the activity of phosphodiesterase in human fibroblasts and in cultured HTC hepatoma cells.

While the present study is only a preliminary one, and indirect in its approach, the ability to distinguish in the human lymphocyte different degrees of responsiveness to theophylline as well as glucocorticoids suggests there may be a genetic variation in some component of the cyclic AMP system. Such a genetic variation in the cyclic AMP system could play a role in the differential ocular response to glucocorticoids and thus perhaps in open-angle glaucoma. Additional studies are underway to explore this hypothesis more directly.

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**REFERENCES**


