Altered Cortisol Metabolism in Cells Cultured from Trabecular Meshwork Specimens Obtained from Patients with Primary Open-angle Glaucoma

A. Louis Southren, Gary G. Gordon, Pedda R. Munnangi, Jozef Vittek, Jerome Schwartz, Carl Monder, Michael W. Dunn, and Bernard I. Weinstein

Cells cultured from trabecular meshwork specimens obtained from patients with primary open angle glaucoma (TMPOAG cells) exhibited two major differences in cortisol-metabolizing enzymes when compared with similar cells from nonglaucomatous patients. One is a marked increase (greater than 100-fold) in Δ^4-3-oxido-reductase activity and the other is a decrease (4-fold) in 3-oxido-reductase activity. Peripheral lymphocytes from one of these patients as well as from five additional patients with POAG, did not show these abnormalities, indicating that the defects are not found in all cortisol-metabolizing cells. The abnormal metabolism of cortisol by TMPOAG cells may be of significance in the pathogenesis of POAG.


Primary open angle glaucoma (POAG) is the most common form of glaucoma and a major cause of blindness. Most studies indicate that the elevation of intraocular pressure is a consequence of impaired aqueous humor outflow through the trabecular meshwork. Patients with this disorder show a marked increase in intraocular pressure following topical or systemic administration of glucocorticoids. It has been suggested that this disease is caused by an abnormal sensitivity to endogenous glucocorticoids (for review, see ref. 4), although no biochemical abnormality has yet been identified in ocular tissues. The present study provides evidence for an alteration(s) in cortisol metabolism in cells cultured from trabecular meshwork specimens obtained from patients with POAG. This metabolic alteration may be of significance in the pathogenesis of this disorder.

Materials and Methods. Trabecular meshwork tissue: Trabecular meshwork was dissected free from sclera and cornea with the aid of a dissecting microscope under sterile conditions from six pairs of eyes obtained from autopsies (41-, 51-, 52-, and 68-year-old women, and two 61-year-old men). The eyes were enucleated within 12 hours of death and processed from 1 to 36 hours later. Histologic examination of the optic discs confirmed the absence of POAG. Trabecular meshwork specimens from three pairs of eyes were used as a source for culturing nonglaucomatous trabecular meshwork cells (TMnOnPOAG cells). The trabecular meshwork from the other eyes were used for metabolism studies.

Surgical trabeculectomy specimens* removed for clinical indications from two patients (women ages 55 and 74 years) with POAG were placed immediately in 5 ml of Dulbecco's Modified Eagle Medium (DMEM) containing antibiotics (penicillin, streptomycin, and kanomycin). Trabecular meshwork was dissected free from sclera and cornea as above and used as a source for culturing trabecular meshwork cells from POAG patients (TMPOAG cells). Each patient had chronic elevation (>21 mmHg) of intraocular pressure, wide open angle by gonioscopy, pathologic cupping of the optic nerve, and typical glaucomatous visual field changes.

Peripheral lymphocytes: Peripheral lymphocytes were obtained from six patients (two women and four men, aged 50 to 86 years) with well-documented POAG as above and three nonglaucomatous volunteers (two men and one woman, aged 40 to 60 years). The lymphocytes were separated from venous blood by sedimentation in a discontinuous Ficoll-Paque® Gradient (Pharmacia). The cells at the interface were removed with a pipette, sedimented, and the pellet washed two times with a glucose-balanced salt solution (1.45 \times 10^{-2} M Tris 1.26 \times 10^{-1} M NaCl, 5 \times 10^{-4} M KCl, 10^{-4} M MgCl_2, 5 \times 10^{-6} M CaCl_2, and 0.01% glucose, pH 7.6). The final pellet was resuspended in Heps Buffered RPMI-1640 medium and used for metabolism studies.

Culture of cells from trabecular meshwork tissue: Dissected trabecular meshwork tissue from autopsy and surgical specimens was placed in 25-cm² plastic tissue culture flasks containing 0.8 ml of DMEM supplemented with newborn calf serum (10%) and antibiotics. The flasks were incubated at 37°C in an atmosphere of 10% CO_2 and 100% relative humidity. After 7 days, the medium was changed twice weekly. Outgrowth occurred from the explant within 2 to 6 weeks. As the outgrowth increased, the volume of medium was increased to 1-1.3 ml.

For subculture, the cells were detached with trypsin/EDTA and the explant was removed and discarded. The cell suspension was diluted 2 to 1. Subsequent passages were carried out at a dilution of 2:1 to 4:1.
In these subcultures the cells have large, flattened epithelioid morphologic features with prominent nucleoli resembling the cultured trabecular meshwork cells described by Polansky et al and Worthen and Cleveland.

Figure 1 shows the cells in culture derived from trabecular meshwork specimens from two patients with POAG (A and B) and from a nonglaucomatous patient at autopsy (C). All three lines are clearly distinguishable from scleral (D) fibroblasts.

**Metabolism of $^3$H cortisol by trabecular meshwork explants, cells in culture and peripheral lymphocytes:**

1. Trabecular meshwork explants were incubated in sterile plastic test tubes containing 1.2, $^3$H-cortisol (S.A. 60 Ci/mmol) in Hepes Buffered Hanks Balanced Salt Solution (HBSS). Approximately 0.1 ml of HBSS was necessary to cover the tissue.

2. The TMnonPOAG and TMPOAG cells to be assayed were grown in 60-mm tissue culture petri dishes to near (80 to 100%) confluence. The amount of protein/dish was determined by the method of Lowry using BSA as standard.

3. Lymphocytes were incubated in sterile plastic test tubes with 0.1-0.5 ml of the labeled cortisol in RPMI-1640. All incubations were at 37°C in an incubator without CO$_2$. Control incubations contained labeled cortisol without tissue or cells. At the indicated times, the steroid-containing incubation medium was removed, the cell layer washed two times with HBSS, and 1.3 to 2 ml of $^3$H-cortisol in HBSS were added.

The steroid-containing incubation medium was removed from the tissue/cells and transferred to borosilicate glass tubes. The residual cell layer or tissue was washed two times with Dulbecco's phosphate-buffered saline and the washes combined with the incubation medium. The steroids were extracted two times from the combined medium and washes with $\frac{1}{2}$ volume ethyl acetate and pooled organic phases evaporated under filtered air or N$_2$ flow. The residue was redissolved in 200 µl benzene:ethanol (9:1 v/v). A 10-µl aliquot was taken for radioactive counting, and an aliquot containing approximately 100,000 cpm was co-chromatographed with a mixture of non-radioactive standard steroids, 20 µg each. Two-dimen-

---

**Generic Names of Steroids**

- Cortisol = 11β,17α,21-trihydroxy-4-pregnene 3,20-dione
- Dihydrocortisol = 11β,17α,21-trihydroxy-5α-pregnane 3,20-dione
- Tetrahydrocortisol = 3α,11β,17α,21-tetrahydroxy-5α-pregnane-20-one
- Cortisone = 17,21-dihydroxy-4-pregnene 3,11,20-trione

---

**Fig. 1.** Phase contrast photomicrographs of human ocular cells in culture derived from dissected surgical and autopsy specimens. A and B are trabecular meshwork cells in the seventh and ninth passage, respectively, derived from trabeculectomy specimens from each of two patients with POAG. The cell in C are trabecular cells in the third passage derived from a nonglaucomatous autopsy eye. D shows scleral fibroblasts in the fifth passage derived from a nonglaucomatous eye. Changes associated with in vitro senescence, such as spreading of the cytoplasm and multinucleated cells, are seen in panels A and B. Bars = 100 μm.
sional thin layer chromatography (TLC) was performed on 20 × 20 cm aluminum sheets (precoated with silica gel 60, F-254 with fluorescent indicator), using the following systems: benzene:acetone (3:2 v/v) and chloroform:acetone (7:3 v/v). The area corresponding to the steroid standards were eluted with methanol and counted in a liquid scintillation counter. Recovery of radioactivity in the various chromatographic fractions accounted for 92–96% of the labeled steroid added to the incubation medium.

**Identification of metabolites:** Identification of the major metabolite was confirmed by crystallization to constant specific activity and derivative formation (acetylation).

**Results and Discussion.** Figure 2 shows the time course of metabolism of 3H-cortisol in a TM nonPOAG and a TM POAG cell line. As can be seen (Fig. 2B), the TM nonPOAG cells metabolized cortisol to cortisone and tetrahydrocortisol (THF) with no dihydrocortisol (DHF) being detected. The conversion of cortisol to cortisone is the result of 11-oxidoreductase activity. The conversion of cortisol to THF requires both Δ^4-reductase (forming DHF) followed by 3-oxidoreductase activity. As is usually the case, the intermediate DHF is not seen. The other three TM nonPOAG cell lines showed a similar pattern of metabolism with cortisone and THF formed but no DHF detected. By contrast, the TM POAG cell line (Fig. 2A) showed a markedly increased rate of metabolism of 3H-cortisol forming large amounts of DHF. The different time scale should be noted. The formation of this metabolite was linear for 4 hours under the conditions of the incubation. Smaller amounts of cortisone also were produced. With longer incubation times, THF was detected. The other TM POAG cell lines showed a similar pattern of metabolism with cortisone and THF formed but no DHF detected. The DHF produced by the TM POAG cell lines, which was tentatively identified by TLC with an authentic standard steroid, was analyzed further by crystallization to constant specific activity (Table 1) and derivative formation (acetylation). The results indicated that the DHF formed consisted of 80% 5α-DHF and 20% 5β-DHF.

The formation of DHF as a function of cortisol concentration in one of the TM POAG cell lines is shown in Figure 3. A Lineweaver-Burke plot appears as an insert. The apparent Km for cortisol is 7.7 × 10^-8 M, and the Vmax is 20 picomoles of DHF formed/mg cell protein/hour. The other TM POAG cell lines showed similar results, with an apparent Km of 12 × 10^-8 M and a Vmax of 20 picomoles of DHF formed/mg cell protein/hour.

Table 1. Sequential recrystallization of 5β-dihydrocortisol*

<table>
<thead>
<tr>
<th>Specific activity (dpm/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated</td>
</tr>
<tr>
<td>Crystallization from acetone and hexane</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

* Pooled fractions corresponding to 5α and 5β-DHF were mixed with 30 mg of nonradioactive steroid standard (5β-DHF) and sequentially crystallized from acetone and hexane as shown in the table. The percent purity was calculated from remaining constant specific activity after the third crystallization, as compared with the calculated specific activity before the first crystallization. It was found that this fraction consists of about 80% 5β-DHF.
Table 2. Distribution of cortisol metabolites after incubation of $^3$H-cortisol with nonglaucomatous trabecular meshwork explants and peripheral lymphocytes from nonglaucomatous and POAG patients

<table>
<thead>
<tr>
<th>Tissue/patient number</th>
<th>Metabolites formed (% of added radioactivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortisone</td>
</tr>
<tr>
<td>Trabecular meshwork*</td>
<td></td>
</tr>
<tr>
<td>(nonglaucomatous)</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes†</td>
<td></td>
</tr>
<tr>
<td>(nonglaucomatous)</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes† (POAG)</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td></td>
</tr>
<tr>
<td>12.†</td>
<td></td>
</tr>
</tbody>
</table>

* Incubation with $10^{-8}$ M cortisol for 25, 36, and 46 hours for patients number 1, 2, and 3, respectively.
† Incubation with $10^{-7}$ M cortisol for 46 to 48 hours.
ND = Not detectible (<0.5%).
‡ Same patient whose TM cells in culture metabolized cortisol to DHF (Fig. 2A).

Control incubations included $^3$H-cortisol and $^3$H-DHF without cells at 37°C and $^3$H-cortisol with cells at 0°C. In all cases, there was no metabolism of the labeled steroids. This illustrates the stability of the $^3$H-steroids during incubation, subsequent extraction and analysis, as well as the requirement of cellular metabolic activity for the metabolism of cortisol. The use of a more nutritious incubation medium such as DMEM with and without newborn bovine serum, successive passages, or varying cell density did not alter the pattern of metabolism seen.

Table 2 shows the conversion of labeled cortisol by trabecular meshwork explants and peripheral lymphocytes isolated from both glaucomatous and nonglaucomatous patients. The three explants all showed the conversion of cortisol predominantly to cortisone and to a lesser extent to THF. There was no DHF detected. This is similar to the pattern of metabolism seen with TM nonPOAG cell lines after four to 11 passages in culture. There was no relationship found between time of processing and the metabolism seen. The peripheral lymphocytes from both the POAG and nonPOAG patients all metabolized cortisol only to tetrahydrocortisol. There was no detectable DHF in or decreased affinity for DHF since the experiment was not done at saturating concentrations of substrate. The large amount of $^3$H-DHF required for measurement of enzyme activity at saturation is not available at present.

Fig. 3. The rate of formation of dihydrocortisol (DHF) as a function of cortisol concentration in a trabecular meshwork cell line derived from a patient with primary open angle glaucoma. The Lineweaver-Burke plot of this data is shown as an insert. TMPOAG cells in petri dishes were incubated for 4 hours with 1.5 ml of medium containing the indicated concentration of $^3$H-cortisol. Each petri dish contained approximately 0.17 mg of cell protein.
either group of patients. It should be noted that one of the patients with POAG, whose lymphocytes were studied, is the same individual whose trabecular meshwork cells in culture metabolized cortisol predominantly to DHF. These data indicate that the alteration in cortisol metabolism in the TM_{POAG} cells is not found in all cortisol metabolizing tissues.

Thus, the TM_{POAG} cells exhibit two alterations in cortisol metabolism. One is a marked increase (greater than 100-fold) in Δ4-reductase activity and the other is a decrease (4-fold) in 3-oxidoreductase activity. The relevance of this observation to the pathophysiology of POAG will depend upon the prevalence of these defects when a larger number of cells lines cultured from trabeculectomy specimens (and trabecular meshwork tissue) derived from POAG patients are evaluated. Future studies will examine the effect of age, sex, and time of processing of the specimens, as well as the identification of the TM_{POAG} cell lines using electron microscopy. The finding that the enzyme defects persist through many generations demonstrates its heritability in culture. Whether the heritable defect is caused by an inborn genetic error or results from effects of chronic ocular hypertension or prior drug therapy remains to be determined. Studies with cells cultured from trabecular meshwork specimens from patients with secondary glaucoma may help to resolve this question.

This study is the first demonstration of a defect in cortisol metabolism in cells obtained from human outflow pathway tissue in POAG.

Key words: cortisol metabolism, trabecular meshwork cells in culture, primary open angle glaucoma, dihydrocortisol

From the Department of Medicine, Section of Endocrinology and Metabolism and the Department of Ophthalmology, New York Medical College, New York, New York. Supported by Grant EY-01313 of the National Eye Institute, National Institutes of Health, Bethesda, Maryland. Submitted for publication: January 4, 1983. Reprint Requests: A. Louis Southren, M.D., Department of Medicine, New York Medical College, Valhalla, NY 10595.

Dr. Carl Monder—Population Council, Rockefeller University, 1230 York Avenue, New York, New York, 10021.

References


Episderal Venous Pressure: A Comparison of Invasive and Noninvasive Measurements

Douglas E. Gaasterland and Jonathan E. Pederson

Noninvasive (pressure chamber) measurements of episcleral venous pressure were followed by invasive (direct cannulation) measurements at the same venous site in seven eyes of four anesthetized rhesus monkeys. There were three definite effects on the vein caused by the pressure chamber that could be used as endpoints: (1) slight indentation; (2) intermittent collapse; and (3) sustained collapse of the vein lumen. The mean pressure in the chamber corresponding to these endpoints was 9.9 ± 0.9, 23.5 ± 2.9, and 31.4 ± 4.0 mmHg (±SE), respectively. After the chamber was removed, the pressure in the veins determined by cannulation measurements at the same sites was 11.3 ± 0.5 mmHg (±SE). Therefore, the first endpoint with the pressure chamber (slight indentation) correlates most closely and slightly underestimates the cannulated pressure. Endpoints defined by partial or complete venous collapse overestimate the venous pressure. Simultaneous measurements with the chamber and a cannula show a rise of local venous pressure caused by the chamber. Invest Ophthalmol Vis Sci 24:1417–1422, 1983.

It is widely accepted that episcleral venous pressure is within the range of 9–11 mmHg in almost all normal