Altered Peripheral Sensitivity to Glucocorticoids in Primary Open-Angle Glaucoma

John Stokes,1,2 Brian R. Walker,1 Jill C. Campbell,1 Jonathan R. Seckl,1 Colm O’Brien,3 and Ruth Andrew1

PURPOSE. Increased levels of glucocorticoids are associated with raised intraocular pressure (IOP). The activity of glucocorticoids is regulated at a prereceptor level by 11β-hydroxysteroid dehydrogenases (11β-HSD). This study was an investigation of the central and peripheral sensitivity to glucocorticoids in patients with POAG or ocular hypertension (OHT) and the differential metabolism of glucocorticoids by 11β-HSDs.

METHODS. Patients with POAG or OHT and normal control subjects were studied. Peripheral sensitivity to glucocorticoids was assessed as dermal blanching and central sensitivity by dexamethasone suppression testing. Daily production rates of glucocorticoids were determined by quantifying metabolites in 24-hour urine. Plasma cortisol levels were determined at baseline (9 AM) and after an overnight low-dose dexamethasone suppression test. In a separate study, plasma and aqueous humor cortisol levels were determined in patients with POAG and normal subjects.

RESULTS. Patients with POAG exhibited a greater cutaneous vasocostricitor response to glucocorticoids than patients with OHT and normal subjects (20.7 ± 3.1 vs. 8.5 ± 4.4 and 8.6 ± 4.5 arbitrary units, respectively; P < 0.05 in each case). Total glucocorticoid production rates were not different between groups, nor were total circulating cortisol levels before or after suppression of the hypothalamic-pituitary-adrenal axis by dexamethasone or concentrations in aqueous humor. The ratio of urinary cortisol to cortisone metabolites was elevated in POAG versus normal control and OHT (1.74 ± 0.13 vs. 1.34 ± 0.11 and 1.32 ± 0.14; P < 0.05 in each case), indicating a change in the balance of 11β-HSDs, without a change in other metabolic pathways.

CONCLUSIONS. Patients with POAG exhibit increased peripheral vascular sensitivity to glucocorticoids. Increased sensitivity of glucocorticoid receptors, may enhance local glucocorticoid action in the eye and exacerbate the adverse effects of glucocorticoids in this condition (Invest Ophthalmol Vis Sci. 2003;44:5163-5167) DOI:10.1167/iovs.02-1318

Ocular or systemic administration of glucocorticoids (GCs) leads to an increase in intraocular pressure (IOP) in susceptible individuals, largely through reduced aqueous outflow. Increased IOP is also observed in patients with excess cortisol secretion (Cushing’s syndrome).1 If GC-induced elevated pressure remains untreated, it may produce optic disc cupping and visual field loss similar to POAG.2 Enhanced ocular sensitivity to GCs, termed steroid responsiveness, occurs in a proportion of the normal population (30%–35%),3,4 and these subjects have an increased risk of development of POAG in later life.5 The proportion of subjects who are steroid responsive is higher in patients with POAG6 and their first-degree relatives7 than in the general population. These observations have raised suggestions of a role for endogenous GCs in the pathogenesis of POAG, but the mechanisms whereby GCs alter IOP are not fully understood, nor indeed are the reasons that some individuals are more sensitive to the effects of GCs than others.

A few studies, reported a number of years ago, have examined biosynthesis of and sensitivity to GCs in patients with POAG, with conflicting results. Patients with POAG and ocular hypertension (OHT) may have increased total plasma cortisol levels,10,11 increased plasma free cortisol,12 and increased cortisol levels in aqueous humor,13 compared with normal subjects. Schwartz et al.10 found that subjects with POAG display impaired suppression of the hypothalamic-pituitary-adrenal (HPA) axis by dexamethasone compared with normal subjects, whereas Rosenberg and Levene14 found that suppression was enhanced. Foon et al.15 and Bigger et al.16 found an increased cellular sensitivity to GCs in patients with POAG, as determined by steroid inhibition of lymphocyte transformation. These important issues merit reexamination with modern techniques.

GCs exert their actions through two receptors, glucocorticoid (GR) and mineralocorticoid (MR), and their effects at these sites are controlled by ligand affinity and efficacy and access of steroid to the receptor. The endogenous GC hormone in humans is cortisol, which binds with high affinity to both GRs and MRs. The concentration of cortisol at the receptor is determined in part by steroid passing into the aqueous humor and ocular tissues from the circulation and also by local regeneration and inactivation by the isozymes of 11β-HSD.

11β-HSD2, an 11β-dehydrogenase, is typically colocated with MRs and inactivates cortisol, yielding inert cortisone. This prevents the access of cortisol to otherwise nonselective MRs, at least in the distal nephron, colon, and salivary gland, allowing aldosterone preferential access.17 11β-HSD1, in contrast, is a predominant 11β-reductase and reactivates circulating cortisone to cortisol, often close to GR.18 Studies have demonstrated the presence of GC target receptors and 11β-HSD1 and -2 in human and mammalian ocular tissues.19,20 Aberrant activity of these enzymes may result in loss of destruction and/or amplification of local GC-mediated events. Differential activity of these enzymes may be responsible for the varying response to endogenous and some exogenous (substrate) steroids in the general population, as dysfunctions in these enzymes have been associated with other features potentially attributable to
function tests, depression, or a history of having taken corticosteroid treatment.

Patients with a history of POAG or OHT were included on the basis of IOP less than 21 mm Hg, normal optic disc appearance, and no family history of POAG. IOP measurements were obtained from patients with POAG or OHT while they were receiving treatment with topical agents. Patients with a history of GC excess (e.g., hypertension, insulin resistance, and obesity).21–25

Our purpose was to perform a comprehensive study of GC secretion and sensitivity in subjects with POAG, to assess whether these subjects were more responsive to GCs in tissues other than the eye and whether POAG was associated with alterations in local activation and inactivation of GCs by 11β-HSDs.

Materials and Methods

Study 1: Measurement of Cortisol Levels in Plasma and Urine, Dexamethasone Suppression Testing, and Skin Blanching

All subjects in this arm of the study were men. Permission was obtained from the Lothian Region Ethics Committee, and written consent was obtained. All studies adhered to the Declaration of Helsinki. All subjects were recruited from the outpatient department of the Princess Alexandra Eye Pavilion, Edinburgh, and are described in Table 1. IOP was obtained according to methods reported previously.22 This method was adapted to allow quantitation of cortisol in aqueous humor as follows: aqueous humor (diluted 1 in 25, 75% ethanol; Sigma-Aldrich, Poole, UK) were placed at each site and the diluent allowed to evaporate. The forearm was then wrapped in clear plastic wrap, and a further outer layer of elasticated bandage to enhance percutaneous absorption of steroid. These coverings remained in place for 16 to 18 hours and were removed 1 hour before measurement of the cutaneous vasoconstrictor response. The degree of blanching was assessed with a reflectance spectrophotometer (Diastron, Andover, UK). Each test site was measured twice, and an average value calculated for each.

The ratio of this value over that of the vehicle response in each patient was calculated and deducted from 1. This was expressed as a percentage and thereafter referred to as the blanching index. The sum of the blanching indices for the five concentrations is presented in the results.

Study 2: Measurement of Plasma and Aqueous Humor Cortisol Levels

Aqueous humor was obtained from male and female patients with POAG (n = 29) who were undergoing trabeculectomy and from male and female control subjects (n = 23) who were undergoing cataract extraction (Table 1). Exclusion criteria were the same as for study 1. Plasma was prepared from blood sampled at 9 AM on the day of surgery for measurement of basal cortisol. Aqueous humor was sampled by performing a paracentesis before commencement of the operative procedure. Samples were obtained between 9 AM and midday.

Biochemical Analyses

Cortisol in plasma was quantified by in-house RIA.22 This method was adapted to allow quantitation of cortisol in aqueous humor as follows: aqueous humor (diluted 1 in 25, 75 μL) was incubated with primary antibody, using a dilution resulting in 20% binding. Urinary steroid metabolites were quantified by gas chromatography mass spectrometry according to methods reported previously.23 Cortisol-binding globulin (CBG) levels were measured by RIA (BioSource, Nivelles, Belgium). Body mass index (BMI) was calculated from measurements of subjects’ heights and weights. Waist-to-hip ratio was calculated from waist measurements taken at umbilical level and hip measurements taken at the level of the greater trochanter. Fasting blood glucose was measured by the hexokinase method.

Statistics

Data from study 1 were compared by ANOVA with post hoc least-significant difference (LSD), and data from study 2 were compared by

<table>
<thead>
<tr>
<th>Study 1</th>
<th>Study 2</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>70.5 ± 1.6</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>27.3 ± 0.7</td>
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<tr>
<td>Systolic BP (mm Hg)</td>
<td>138.0 ± 4.8</td>
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<tr>
<td>Diastolic BP (mm Hg)</td>
<td>79.0 ± 2.9</td>
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<tr>
<td>HbA1c (%)</td>
<td>6.2 ± 0.1</td>
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<tr>
<td>IOP (mm Hg)</td>
<td>17.2 ± 0.7</td>
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</table>

Data are the mean ± SEM.

* P < 0.05, for POAG versus normal subjects.
† P < 0.05, for POAG versus OHT.
DISCUSSION
Previous studies have examined the circulating levels of GCs and control of their biosynthesis by the HPA axis and found contrasting results. Our results suggest that neither the circu-

Table 2. Cortisol Concentrations and Associated Factors in Plasma and Serum

<table>
<thead>
<tr>
<th>Study 1</th>
<th>POAG</th>
<th>OHT</th>
<th>NORMAL</th>
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</thead>
<tbody>
<tr>
<td>Cortisol (plasma, 9 AM, nM)</td>
<td>569.0 ± 85.0</td>
<td>407.0 ± 52.0</td>
<td>504.0 ± 75.0</td>
</tr>
<tr>
<td>Cortisol (post DST, nM)</td>
<td>264.0 ± 38.0</td>
<td>192.0 ± 23.0</td>
<td>211.0 ± 34.0</td>
</tr>
<tr>
<td>Blushing index (arbitrary units)</td>
<td>20.7 ± 3.1</td>
<td>8.5 ± 4.4</td>
<td>8.6 ± 4.5</td>
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<tr>
<td>Study 2</td>
<td></td>
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<tr>
<td>Cortisol (plasma, 9 AM, nM)</td>
<td>416.0 ± 31.0</td>
<td>425.0 ± 40.0</td>
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<tr>
<td>CBG (μM)*</td>
<td>4.07 ± 0.94</td>
<td>4.54 ± 0.04</td>
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<tr>
<td>Unbound cortisol (nM)</td>
<td>39.1 ± 4.7</td>
<td>45.6 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>Cortisol (aqueous humor, nM)</td>
<td>28.7 ± 6.5</td>
<td>27.0 ± 1.5</td>
<td></td>
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</tbody>
</table>

Data are the mean ± SEM.
* P ≤ 0.05, POAG versus normal.
† P < 0.05, POAG versus normal.

Student’s t-test. Variables that were not normally distributed (cortisol concentrations in plasma and aqueous humor, total GC metabolites, cortisol to cortisone [F/E] ratio, and the tetrahydrocortisol-to-allotetrahydrocortisol [THF/αTHF] ratio), were transformed logarithmically before statistical analysis.

RESULTS

Subject Characterization

In study 1, subjects with POAG were somewhat older than those in the other two groups, but systolic and diastolic blood pressure and BMI did not differ between groups. Patients of POAG had slightly higher hemoglobin A1c (HbA1c) levels, although this was in the normal range in all cases (5%-6.5%). Subjects in study 2 were age matched, with no difference in diastolic blood pressure or HbA1c. However, they showed a slightly lower systolic blood pressure.

Dermal Sensitivity to GCs

Cutaneous vasoconstriction in response to topical GCs was enhanced in subjects with POAG compared with that in those with OHT (P < 0.05) and normal subjects (P < 0.05; Table 2).

HPA Axis Sensitivity

Basal cortisol concentrations were not different in the two groups of patients compared with the control subjects. Feedback on the HPA axis, as determined by a low-dose dexamethasone suppression test, was not different between groups (Table 2). To determine total daily GC production more accurately, we examined total amounts of GC metabolites appearing in urine over 24 hours (Table 3). The levels were the same in subjects with POAG versus OHT and normal control subjects.

Table 3. Glucocorticoid Metabolites from Urine of Subjects with POAG or OHT and Normal Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>POAG</th>
<th>OHT</th>
<th>NORMAL</th>
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</thead>
<tbody>
<tr>
<td>THF (mg/24 h)</td>
<td>3.53 ± 0.70</td>
<td>2.56 ± 0.30</td>
<td>3.08 ± 0.60</td>
</tr>
<tr>
<td>αTHF (mg/24 h)</td>
<td>3.58 ± 0.50</td>
<td>2.57 ± 0.40</td>
<td>4.28 ± 1.00</td>
</tr>
<tr>
<td>THE (mg/24 h)</td>
<td>4.46 ± 0.50</td>
<td>4.26 ± 0.50</td>
<td>6.26 ± 1.60</td>
</tr>
<tr>
<td>Total urinary glucocorticoids (mg/24 h)</td>
<td>15.40 ± 1.70</td>
<td>12.10 ± 1.40</td>
<td>18.10 ± 4.20</td>
</tr>
<tr>
<td>THF+αTHF/THE (11β-HSD index)†</td>
<td>1.74 ± 0.13</td>
<td>1.52 ± 0.14</td>
<td>1.34 ± 0.11</td>
</tr>
<tr>
<td>F/E (renal 11β-HSD2 index)</td>
<td>1.66 ± 0.38</td>
<td>1.25 ± 0.15</td>
<td>1.07 ± 0.07</td>
</tr>
<tr>
<td>5βTHF/5αTHF</td>
<td>1.18 ± 0.20</td>
<td>1.02 ± 0.10</td>
<td>0.90 ± 0.10</td>
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</tbody>
</table>

Data are the mean ± SEM.
* P < 0.05, POAG versus normal.
† P < 0.05, POAG versus OHT.

Indices of Metabolic Enzymes

The ratio of cortisol to cortisone, an index of renal 11β-HSD 2,25 was not altered between groups (Table 3). The ratio of metabolites of cortisol to those of cortisone was significantly higher in subjects with POAG than in those with OHT (P < 0.05) and the normal control subjects (P < 0.05). This is an index of increased 11β-HSD1 activity,25 in the absence of a change in the cortisol/cortisone ratio. The ratio of 5α- to 5β-reduced metabolites of GCs (measuring 5α- and 5β-reductions, the other major routes of cortisol metabolism) did not differ between groups. Age did not correlate with total GC levels or in the ratio of tetrahydrocortisol+allotetrahydrocortisol to tetrahydrocortisone (THF+αTHFs/THE) in the groups and thus the difference in age between the groups did not account for the differences observed.

Cortisol in Blood and Aqueous Humor

Concentrations of cortisol in plasma (total and unbound, 9 AM) and aqueous humor (9 AM to midday) were not different between groups. However in patients with POAG, plasma CBG levels were higher than in normal subjects (P = 0.02; Table 2). Plasma total cortisol and free cortisol did not correlate with levels in aqueous humor. There were no differences in any of these parameters between men and women; therefore, subjects were analyzed as a group (CBG, men versus women P = 0.99, aqueous humor cortisol, men versus women P = 0.21, plasma cortisol, men versus women P = 0.79).

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lating amounts, even when adjusted for plasma binding, nor the central sensitivity of the HPA axis are aberrant in POAG. In contrast, this study demonstrates that subjects with POAG are more sensitive to GCR-mediated effects in the peripheral vasculature, in addition to the enhanced ocular sensitivity documented previously. The overall balance of 11β-HSDs in subjects with POAG, suggest that cortisol concentrations are favored over those of cortisone which, if this were also reflected in the eye, may expose patients to greater local levels of active GCs. Thus, despite normal circulating levels of cortisol, patients with POAG appear to exhibit two separate mechanisms capable of producing increased GC action.

Patients with POAG demonstrated an increased cutaneous vasoconstrictor response to GCs. This test provides an index of peripheral GC receptor sensitivity in conditions such as asthma, in which there is a range in responsiveness across the population. The increased GR responsiveness observed in POAG is in agreement with the data of Foon et al.15 and Bigger et al.,16 who used lymphocyte transformation as an index of GC sensitivity. Panarelli and Fraser27 found a correlation between lymphocyte transformation and systemic vasoconstriction as an index of GC sensitivity in systemic hypertension. Ebrecht et al.28 did not find a correlation between these indices in persons with systemic hypertension.

Changes in the trabecular meshwork are characteristic of POAG and steroid administration, with outflow facility being impaired as a likely consequence of poorer flexibility of the network and deposition of proteins in the drainage spaces. In cultured trabecular meshwork cells, activation of GR by GCs induces changes in the arrangement of microfilaments, resulting in a more rigid structure.29,30 which is thought to in part reduce outflow of aqueous humor. GCs also inhibit activity of metalloproteinases31,32 and stimulate deposition of extracellular matrix proteins, such as fibronectin33 and laminin.34 These GC effects in cell culture are consistent with accumulation of plaque material in the trabecular meshwork of GC-treated eyes35 and glaucomatous eyes36 in the cytosol and extracellular matrix of human trabecular meshwork cells, which also reduces the drainage capacity. If the enhanced sensitivity of GR demonstrated in the periphery is paralleled in the tissues of the eye, it may promote ocular disease. Nevertheless, increased GC sensitivity cannot be generalized, because, first, sensitivity to GC feedback on the HPA axis was normal and, second, plasma CBG, a liver gene product usually negatively regulated by GCs, was actually higher in patients with POAG, thus providing no suggestion of increased hepatic GC sensitivity.

Enhanced vasoconstriction in response to GCs may be important in its own right, because vascular risk factors and blood flow have been postulated to play roles in the pathologic course of certain forms of glaucoma, such as normal-tension glaucoma. Buckley et al.37 showed an enhanced contractile response in resistance arteries of these patients to endothelin and 5-HT. Whether patients with POAG also show an enhanced vasoconstrictor response to GCs in their ocular vasculature remains to be shown.

The ratio of cortisol to cortisone and the ratio of their metabolites are accepted indices of the activities of the 11β-HSDs in vivo. Both isozymes are important in the regulation of GC hormones to their receptors, and disruption in either system may influence local active cortisol levels. In subjects with POAG, the balance of cortisol and cortisone isozyme expression favored the active hormone; however, it is not clear which isozyme was responsible for the changes observed. The ratio of F/E (cortisol to cortisone) is regarded as an index of renal 11β-HSD2, and there was a weak trend toward a higher ratio in patients with POAG. The importance of 11β-HSD1 in amplifying GC action is exemplified in mice bred with transgenic overexpression of this gene in adipose tissue. Such mice produced increased active GCs in fat and have central obesity, which develops through GR-mediated lipid accumulation, diabetes, and hyperlipidemia. In addition overexpression of this enzyme in liver results in insulin resistance and fatty liver, again a consequence of enhanced action of GCs. The incidence of POAG is in agreement with the data of Foon et al.15 and Bigger et al.,16 who used lymphocyte transformation and systemic vasoconstriction as an index of GC sensitivity in systemic hypertension. Ebrecht et al.28 did not find a correlation between these indices in persons with systemic hypertension.

Earlier studies of cortisol pharmacokinetics in this field did not distinguish between patients with POAG (elevated IOP with glaucomatous damage) and OHT (elevated IOP with normal visual fields).10,13,14 Patients with OHT represent a heterogeneous group comprising individuals who may never have POAG, despite increased IOP, and those who will have glaucoma-induced damage. Furthermore, previous studies did not exclude those with diabetes or hypertension, two conditions that are associated with GC excess21,22 and an increased incidence of POAG.22,43 Of interest, in this study the balance of 11β-HSDs was not elevated in subjects with OHT. Therefore, at the time of the study, they were relatively protected from local effects of GCs, perhaps permitting maintenance of IOP at a higher value, without the accompanying tissue damage that occurs in POAG. Central corneal thickness has been shown to be an important parameter that influences IOP measurement by Goldmann applanation tonometry.42,43 Central corneal thickness was not measured in this study; future studies should include this parameter.

We have demonstrated alteration in several features determining delivery, production and action of cortisol in POAG, which may expose these subjects to subtly increased local activity of GCs. Our study has identified these features mainly in peripheral tissues, and it is important to extend these observations to ocular tissues and fluids. 11β-HSD type 1 is a potential therapeutic target already established as locally regulating GC action. Administration of a specific inhibitor of this enzyme,45,46 as yet unavailable commercially, will give valuable insight into novel therapeutic approaches to treatment of POAG.

Acknowledgments
The authors thank Dan Burt for assistance.

References


