

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 11 β -hydroxysteroid dehydrogenases (11 β -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 11 β -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 vasoconstrictor 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 11 β -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

From the ¹Endocrinology Unit, School of Molecular and Clinical Medicine, University of Edinburgh, Western General Hospital, Edinburgh, Scotland, United Kingdom; the ²Department of Ophthalmology, Princess Alexandra Eye Pavilion, Edinburgh, Scotland, United Kingdom; and the ³Institute of Ophthalmology, Mater Hospital, Dublin, Ireland.

Supported by grants from the British Council for the Prevention of Blindness, The Ross Foundation, The Urquhart Trust, The Royal College of Surgeons of Edinburgh and the Wellcome Trust.

Submitted for publication December 20, 2002; revised May 16, 2003; accepted June 2, 2003.

Disclosure: **J. Stokes**, None; **B.R. Walker**, None; **J.C. Campbell**, None; **J.R. Seckl**, None; **C. O'Brien**, None; **R. Andrew**, None

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Corresponding author: John Stokes, Department of Ophthalmology, Princess Alexandra Eye Pavilion, Chalmers Street, Edinburgh EH3 9HA, UK; jst2121@aol.com.

Ocular or systemic administration of glucocorticoids (GCs) leads to an increase in intraocular pressure (IOP) in susceptible individuals, largely through reduced aqueous outflow.¹ Increased IOP is also observed in patients with excess cortisol secretion (Cushing's syndrome).^{2,3} If GC-induced elevated pressure remains untreated, it may produce optic disc cupping and visual field loss similar to POAG.⁴ Enhanced ocular sensitivity to GCs, termed steroid responsiveness, occurs in a proportion of the normal population (30%-35%),^{5,6} and these subjects have an increased risk of development of POAG in later life.⁷ The proportion of subjects who are steroid responsive is higher in patients with POAG⁸ and their first-degree relatives⁹ 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 11 β -HSD.

11 β -HSD2, an 11 β -dehydrogenase, is typically colocalized 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 11 β -HSD1, in contrast, is a predominant 11 β -reductase and reactivates circulating cortisone to cortisol, often close to GR.¹⁸ Studies have demonstrated the presence of GC target receptors and 11 β -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 isozymes 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

TABLE 1. Characteristics of Patients and Control Subjects

	POAG (<i>n</i> = 23)	OHT (<i>n</i> = 20)	NORMAL (<i>n</i> = 18)
Study 1			
Age (y)*†	70.5 ± 1.6	63.4 ± 2.1	61.2 ± 1.8
BMI (kg/m ²)	27.3 ± 0.7	31.6 ± 2.1	27.7 ± 0.9
Systolic BP (mm Hg)	138.0 ± 4.8	142.0 ± 3.9	134.0 ± 3.8
Diastolic BP (mm Hg)	79.0 ± 2.9	78.0 ± 1.8	78.0 ± 2.3
HbA _{1c} (%)*†	6.2 ± 0.1	5.5 ± 0.1	5.8 ± 0.1
IOP (mm Hg)*†	17.2 ± 0.7	19.6 ± 0.8	15.0 ± 0.5
Study 2			
Age (y)	77.1 ± 2.2		76.1 ± 2.2
Men, women (<i>n</i>)	14, 15		14, 9
Systolic BP (mm Hg)*	114.0 ± 1.9		120.0 ± 1.2
Diastolic BP (mm Hg)	80.0 ± 1.7		80.0 ± 1.6
HbA _{1c} (%)	4.7 ± 0.2		4.7 ± 0.1
IOP (mm Hg)*	19.3 ± 0.9		13.6 ± 0.5

Data are the mean ± SEM.

* *P* < 0.05, for POAG versus normal subjects.

† *P* < 0.05, for POAG versus OHT.

GC excess (e.g., hypertension, insulin resistance, and obesity).²¹⁻²³

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 is presented as the mean pressure in the left and right eyes. Patients with POAG (*n* = 23) were included on the basis of increased IOP (Goldmann) of more than 21 mm Hg (before treatment), optic disc cupping, and abnormal results in a visual field test. Patients with normal-tension glaucoma, angle-closure glaucoma, and secondary glaucomas were not included. Patients with ocular hypertension (*n* = 19) were included on the basis of more than one documented IOP higher than 21 mm Hg and normal visual fields. Normal patients (*n* = 16) 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 diabetes mellitus, systemic hypertension, abnormal results in thyroid function tests, depression, or a history of having taken corticosteroid medication in the previous 3 months were excluded.

Investigations

Plasma (9 AM) was obtained from subjects to determine basal cortisol concentrations. To determine the sensitivity of the HPA axis to suppression by dexamethasone, subjects took dexamethasone (250 μg, orally) on another occasion at 11 PM, followed by an overnight fast. Plasma was prepared from blood sampled at 9 AM the following morning. Urine was collected over 24 hours. Dermal sensitivity to GCs was assessed in a cutaneous vasoconstrictor assay.²⁴ Subjects' forearms were shaved, if necessary, and a cardboard sheet, out of which six

2-cm diameter circular holes were cut, was placed onto the forearm and the edges of the sheet marked out. Test concentrations (10 μL) of beclomethasone dipropionate (1-100 μg/mL, 95% 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.²⁴ 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.²⁵ 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 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 2. Cortisol Concentrations and Associated Factors in Plasma and Serum

	POAG	OHT	NORMAL
Study 1			
Cortisol (plasma, 9 AM, nM)	569.0 ± 85.0	407.0 ± 52.0	504.0 ± 73.0
Cortisol (post DST, nM)	264.0 ± 38.0	192.0 ± 23.0	211.0 ± 34.0
Blanching index (arbitrary units)*†	20.7 ± 3.1	8.5 ± 4.4	8.6 ± 4.5
Study 2			
Cortisol (plasma, 9 AM, nM)	416.0 ± 31.0		425.0 ± 40.0
CBG (μM)*	0.67 ± 0.04		0.54 ± 0.04
Unbound cortisol (nM)	39.1 ± 4.7		45.6 ± 6.1
Cortisol (aqueous humor, nM)	28.7 ± 6.5		27.0 ± 1.5

Data are the mean ± SEM.

* $P \leq 0.05$, 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 (HbA_{1c}) 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 HbA_{1c}. 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.

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-reductases, 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 $P = 0.79$).

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

	POAG	OHT	NORMAL
THF (mg/24 h)	3.53 ± 0.70	2.56 ± 0.30	3.08 ± 0.60
αTHF (mg/24 h)	3.58 ± 0.50	2.57 ± 0.40	4.28 ± 1.00
THE (mg/24 h)	4.46 ± 0.50	4.26 ± 0.50	6.26 ± 1.60
Total urinary glucocorticoids (mg/24 h)	15.40 ± 1.70	12.10 ± 1.40	18.10 ± 4.20
THF+αTHF/THE (11β-HSD index)*†	1.74 ± 0.13	1.32 ± 0.14	1.34 ± 0.11
F/E (renal 11β-HSD2 index)	1.66 ± 0.38	1.25 ± 0.15	1.07 ± 0.07
5βTHF/5αTHF	1.18 ± 0.20	1.02 ± 0.10	0.90 ± 0.10

Data are the mean ± SEM.

* $P < 0.05$, POAG versus normal.

† $P < 0.05$, POAG versus OHT.

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.⁸ The overall balance of 11 β -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.²⁶ 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 metalloproteinases^{31,32} 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 11 β -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 interconversion 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 11 β -HSD2, and there was a weak trend toward a higher ratio in patients with POAG. The importance of 11 β -HSD1 in amplifying GC action is exemplified in mice bred with transgenic overexpression of this gene in adipose tissue. Such mice pro-

duce increased active GCs in fat and have central obesity, which develops through GR-mediated lipid accumulation,³⁸ 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.³⁹ 11 β -HSD1 is expressed in several sites in the eye,^{19,20} including the trabecular meshwork, and enhanced reactivation of cortisol at this site, in conjunction with enhanced receptor responsiveness, may render the patient more sensitive to ocular damage induced by GCs compared with a normal subject.

The inference that the balance of 11 β -HSDs activity may be altered in POAG, amplifying GC action, comes from urinary metabolite ratios. This is an accepted index of whole body enzyme activity, and thus it is important to extend these investigations to look at the cortisol-cortisone ratio in the eye, in that there is evidence in obesity that the regulation of this enzyme may be tissue specific.²³ In this study we were unable to demonstrate an increase in cortisol levels in aqueous humor of subjects with POAG versus control subjects; however, this increase is difficult to interpret because the levels present are influenced by the circulating levels responding to the stress of surgery and also perhaps also to anesthesia. We were unable to perform assays for cortisone in aqueous humor in this study as a result of small sample volumes, but determination of the F/E ratio would be of interest in future studies. Rauz et al.²⁰ reported cortisol levels in excess of those of cortisone in aqueous humor of normal subjects and suggest that this supports a role for active regeneration of cortisol in the eye. IOP decreased in response to administration of an inhibitor of 11 β -HSD1, potentially as a result of inhibition of cortisol generation.²⁰

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.^{40,41} Furthermore, previous studies did not exclude those with diabetes or hypertension, two conditions that are associated with GC excess^{21,22} and an increased incidence of POAG.^{42,43} Of interest, in this study the balance of 11 β -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 influence IOP measurement by Goldmann applanation tonometry.⁴⁴ 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. 11 β -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.

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