Evaluation of Glucocorticoid Sensitivity and Its Potential Clinical Applicability

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Key Words

Glucocorticoid sensitivity • Glucocorticoid receptor • Dexamethasone suppression test

Abstract

Glucocorticoids (GC) play an important role in physiologic and pathophysiologic adaptive responses to stress. The majority of these effects are mediated by the GC receptors (GR). GC sensitivity largely depends of the amount of available GR, and their ability to bind the GC-responsive element and/or other nuclear transcription factors, leading to modulation of the expression of GC target genes. Clinical conditions of tissue-specific GC resistance or GC hypersensitivity have been described in several diseases, such as chronic inflammatory and autoimmune conditions, and in visceral obesity, such as metabolic syndrome. Several in vivo and in vitro methods have been described, allowing the evaluation and quantitation of GC sensitivity. The recognition of these parameters has improved our comprehension of the mechanisms involved in those diseases, with potential implications for the diagnosis and therapy of such abnormalities.

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Introduction

Glucocorticoids (GC) are important modulators in a variety of physiologic systems, and are also a key component involved in the pathophysiology of several diseases

particularly related to abnormal tissue-specific GC resistance or GC hypersensitivity [1].

GC are largely employed as a therapy, as a substitute for cortisol, or because of their anti-inflammatory effects, when given in supra-physiologic doses [2]. In certain cell types, cell death by apoptosis can also be reached with therapeutic doses of GC, which is a relevant effect in the treatment of neoplastic disorders.

Clinical response to GC is extremely variable. This is related to the GC type, dose and schedule, and to the disease or cell type to be targeted; however, it is also dependent on factors modulating each individual's GC sensitivity [3].

Interestingly, patients with GC-dependent disorders are those usually presenting tissue-specific resistance, such as in arthritis and asthma [4–5]. Concomitance of inadequate inflammatory control and excessive effects on the pituitary, bone and other tissues are common findings in these individuals, suggesting variability in cortisol metabolism and action in different tissues [6–7]. Every individual's cellular GC sensitivity is an influential factor, along with the GC receptor (GR) numbers, regulation of splice or translational GR variants, mutations and/or polymorphisms in the GR gene, and the availability of cofactors as important variables [8].

This reinforces the need for recognition of individual variation, and organ- or tissue-specific aspects modulating GC sensitivity. The recognition of this profile can have relevant implications for GC therapy.

Fig. 1. Modulators of GS. HPA = Hypothalamic-pituitary-adrenal axis; CBG = cortisol-binding globulin; 11 β -HSD = 11 β hydroxy-steroid dehydrogenase; GRE = GC-responsive elements (target genes).

Considering that the GC effects are predominantly mediated by the GR, one of the major determinants of GC responsivity is the intracellular density of the active isoform GR α , which is in agreement with the correlation observed between GC sensitivity and the amount of GR determined by several in vitro assays [9].

In this review, we briefly described the multiplicity of factors involved in the signaling cascade of GC action and their influence on GC sensitivity. We also report on the experience gained by our group in the development of in vivo and in vitro methods, which can be helpful in quantifying GC sensitivity in both physiologic conditions, such as physical exercise, and pathologic conditions, such as obesity and rheumatoid arthritis.

Modulators of GC Sensitivity

Significant differences have been observed in GC sensitivity among species, individuals, tissues, or even different cells at a different cell-cycle phase [1]. Several modulators of GC sensitivity have already identified, such as (1) bioactive GC availability, influenced by cortisol-binding globulin and by the activity of 11 β -hydroxysteroid dehydrogenase types 1 type 2; (2) the number, hormonal affinity and phosphorylation pattern of GR; (3) nuclear translocation and the ability to bind and activate the GC-responsive element; (4) interrelationship with the transcription factors, leading to modulation of the transcription of GC target genes [10–13] (fig. 1).

In pathologic conditions, such as trauma, acute or chronic diseases, hypoglycemia and behavioral disorders, cortisol production increases several times [14–16].

In inflammatory diseases, cytokines – such as interleukins 1 and 6, tumor necrosis factor- α and leukemia inhibitory factor – are substantially increased, rendering a GC insensitivity and an interplay among modulators from the endocrine and immune systems [17].

GR mutations and polymorphisms are also determinants of GC sensitivity, influencing the binding affinity to the ligand and the expression rate of the GR gene [18]. Resistance to GC can be generalized or tissue specific. Generalized resistance includes the hypothalamic and the pituitary regions (with a consequent failure in the negative feedback), elevated ACTH concentrations, and increased cortisol secretion. Excessive ACTH stimulation determines higher androgen and mineralocorticoid production with a variable clinical presentation of virilization and salt retention [18]. Clinical manifestations of GC resistance and a description of the mutations in the gene encoding GR are presented in tables 1 and 2, respectively.

Generalized GC hypersensitivity is a rare phenomenon and it is poorly characterized. Iida et al. [19] first reported a patient with symptoms of Cushing's syndrome, despite hypocortisolemia. The molecular etiology of GC hypersensitivity has not been fully clarified, but 2 single nucleotide polymorphisms of the GR gene seem to play an important role in determining hypersensitivity [20]. The N363S polymorphism has been associated with increased sensitivity to GC, lower bone mineral density, and increased BMI [21]; however, other reports found no association with BMI [22]. Another polymorphism (BclI polymorphism), recently identified as a C $\rightarrow$ G nucleotide change, associated the G allele with increased sensitivity to GC [23]. In men, the BclI haplotype was associated with a 34% higher risk of cardiovascular disease [24].

Table 1. Clinical manifestations of GC resistance

Clinical presentation
Apparently normal GC function <ul style="list-style-type: none">AsymptomaticChronic fatigue
Mineralocorticoid excess <ul style="list-style-type: none">HypertensionHypokalemic alkalosis
Androgen excess <ul style="list-style-type: none">Children: ambiguous genitalia at birth, premature adrenarche, precocious pubertyFemales: acne, hirsutism, male-pattern hair loss, menstrual irregularities, oligo-anovulation, infertilityMales: acne, hirsutism, oligospermia, adrenal rests in the testes, infertility
Increased HPA axis activity (CRH/ACTH hypersecretion) <ul style="list-style-type: none">AnxietyAdrenal rests
CRH = Corticotropin-releasing hormone; ACTH = adreno-corticotrophic hormone. Modified from reference 18.

In vivo Evaluation of GC Sensitivity

The in vivo models used to study GC sensitivity were initially directed to detect GC effects, such as salt retention, influence on the carbohydrate metabolism, and anti-inflammatory and pro-apoptotic actions [25–27]. NF- κ B and activator protein 1 also interfere with these GC effects, and should be measured simultaneously [28].

The standard in vivo evaluation is represented by the determination of cortisol suppression after dexamethasone. High doses of dexamethasone have been used for the diagnosis of Cushing syndrome. On the other hand, doses varying from 20 to 80 μ g/kg/day invariably suppress cortisol in all normal subjects, preventing its use in the recognition of normal individual GC sensitivity [29]. We previously reported the use of dexamethasone in a single dose of 75 μ g/m² p.o. in normal and in obese children [30], allowing the detection of GC sensitivity in a physiologic range. In order to exclude the interference on dexamethasone metabolism during the first passage through the liver [31], we subsequently described the use of i.v. bolus administration of dexamethasone in a dose of 20 μ g/m² [32]. This very low dose of i.v. dexamethasone is a useful method for in vivo establishment of GC sensitivity.

We also tested the applicability of this i.v. very-low-dose dexamethasone suppression test (VLD-DST) in

Table 2. Molecular basis and phenotype of GC resistance

Mutation position		
cDNA	Amino acid	Phenotype
1922 (A→T)	641 (D→V)	hypertension hypokalemic alkalosis hirsutism male-pattern hair loss menstrual irregularities
4-bp deletion in exon-intron 6		
2185 (G→A)	729 (V→I)	precocious puberty hyperandrogenism
1676 (T→A)	559 (I→N)	hypertension oligospermia infertility
1430 (G→A)	477 (R→H)	hirsutism fatigue hypertension
2035 (G→A)	679 (G→S)	hirsutism fatigue hypertension
1712 (T→C)	571 (V→A)	ambiguous genitalia hypertension hypokalemia hyperandrogenism
2241 (T→G)	747 (I→M)	cystic acne hirsutism oligo-amenorrhea
2318 (T→C)	773 (L→P)	fatigue anxiety acne hirsutism hypertension
2209 (T→C)	737 (F→L)	hypertension hypokalemia
Modified from reference 18.		

some stressful conditions. In normal individuals submitted to intensive and prolonged physical training, low basal cortisol levels – associated with lower cortisol reduction after the i.v. VLD-DST – are observed after the training program. Together, these observations suggest lower activity of the HPA axis with a reduction in the pituitary negative feedback to dexamethasone, possibly reflecting a more systemic reduction in GC sensitivity [33].

In vitro Evaluation of GC Sensitivity

The methods to perform in vitro measurements of GC sensitivity include determination of glucose or amino acid concentrations and transport, protein synthesis, and

RNA expression assays. Measurements of cell survival, cell proliferations, or cell death are also useful [34].

Considering that GC action is predominantly mediated by GR α [35], several qualitative and semi-quantitative assays were previously described. Initial techniques employed GR-binding capability in intact cells, allowing quantitation of bioactive cytosolic/nuclear GR [36]. Tissue-specific GR gene expression was also described by measuring GR-cDNA obtained from several tissues samples or by Northern blotting analyses [37]. Homologous downregulation of GR is possible by Western blotting assays employing specific anti-GR antibodies [38].

We recently described a method of absolute quantitation of GR α gene expression by real-time PCR [39]. This method is accurate and reproducible, with intra- and inter-assay coefficients of variation of 2 and 7%, respectively. It employs a standardized cell line in the construction of standard curves, and a normalizing gene (BCR). We also showed a relationship between GR α expression and the GC sensitivity measured by the intravenous VLD-DST, especially those cases in which high GR expression was observed (unpublished data).

The effects of intensive and prolonged exercise on gene expression were also evaluated by real-time PCR, showing a significant reduction in GR α and a global decrease

in the expression of inflammatory genes of the IKK/I κ B/NF- κ B pathway, accompanied by a reduction in several interleukins [40].

GR α gene expression in patients with rheumatoid arthritis showed an inverse correlation with GC sensitivity, suggesting that an upregulation of GR α is present in these patients, possibly related to a post-receptor phenomenon [41].

Additional clinical applicability should be evaluated, employing a combination of methods described in this short review. This can contribute to the diagnosis and therapeutic schedule of several diseases presenting variable states of GC sensitivity, like tissue-specific GC resistance described in asthma, rheumatoid arthritis, and lymphoblastic cells [4–5, 38]. In clinical practice, GC is widely used to treat diseases (e.g. asthma, chronic inflammation, prevention of rejection of organ transplants) as well as replacement therapy. It is known that GC effects vary considerably between patients. Some patients respond to therapeutical administration of GC, but also develop side effects; while others need a very high dose to establish clinical effects. This dose should be adjusted to a patient need, considering the GC sensitivity, in such a way that it is therapeutically effective but does not cause side effects.

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