Glucocorticosteroids and the Glucocorticosteroid Receptor in Asthma: Answers for Today and Tomorrow

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INTRODUCTION
Glucocorticosteroids (GC) have a profound influence on human homeostasis and are used extensively in clinical medicine. GC mediate their effects via the glucocorticoid receptor (GR) – a member of the superfamily of ligand-regulated nuclear receptors. Since the cloning of GR in 1985 there have been important developments in the molecular biology of GR that are crucial for improved understanding of asthma pathophysiology and therapeutics.

STRUCTURE AND ACTIVITY OF GR
The GR consists of 3 domains – the amino N-terminal or immunogenic domain and carboxy C-terminal or ligand-binding domain, flanking a DNA-binding domain (DBD). The inactive GR is located in the cytoplasm as a hetero-oligomeric complex containing heat shock proteins 50, 70 and 90 and other proteins as well (Fig.1).

After binding to GC, the GR undergoes conformational changes, dissociating from the chaperone proteins and revealing two nuclear localisation sequences (NL1 & 2) that enable translocation to the nucleus. Here transactivation by GR dimers requires specific palindromic sequences in the cis-regulatory regions of target genes called the GC response element (GRE). This is the mechanism whereby β-adrenoceptor regulation and the inhibitor-I-kBα of the transcription factor NF-kB is controlled. Gene repression is mediated by negative GREs. However, many of the major pro-inflammatory genes do not possess GREs; this suggests that other mechanisms of inhibition exist. Transrepression occurs when GR (probably in its monomeric form) engages in protein-protein interactions without DNA binding, examples include NF-kB and AP-1 binding.

GR-β
One of the major discoveries regarding GR in the last few years, particularly in respect of asthma, has been the recognition of GR-β. Alternative splicing of GR pre-mRNA gives rise to two variants of GR: (i) GR-α, the 777 amino acid moiety and active form of the GR; and (ii) GR-β, which differs only in the carboxy terminal end by being deficient in 35 amino acids. However, being in the crucial ligand-binding domain, this isoform is unable to bind corticosteroids (CS). Interestingly, GR-β is synthesised in normal individuals, but its production is increased in patients with asthma and the highest levels are found in cases of severe and near-fatal asthma. Clearly, this explains why these patients respond poorly to CS treatment – they have a receptor that cannot bind CS and therefore cannot mediate the anti-inflammatory effects! Worse still – GR-β inhibits the GR-α, further diminishing the possibility of a good therapeutic outcome. Some of the mechanisms whereby this occurs include GR-β competitive binding to GRE-DNA and the formation of transcriptionally impaired GR-α GR-β heterodimers instead of the active homodimer. An important line of investigation has opened up regarding the control of GR-β expression and whether this can be influenced beneficially. We do not currently know what these factors are, but what has been demonstrated is that inflammation increases GR-β expression. GR-β is also increased at night, providing...
yet another mechanism and explanation for the characteristic diurnal symptomatology of asthma.

Fortunately GR-β has a negligible effect on the binding of other steroid hormones.

**INFLUENCE OF SIGNAL TRANSDUCTION ON GR**

Cell stimulation results in the activation of intracellular enzyme systems and the generation of a number of signal transduction proteins. These have the capacity to interact with GR and enhance or inhibit its function.1

**GR affinity**

The effectiveness of a ligand in stimulating GC actions is dependent on its ability to bind to the receptor and the number of receptors present. The inability of some patients to respond to GC has been proposed to be due to altered affinity for GR and/or number of receptors. In studies at the Royal Brompton, we were able to demonstrate that GR numbers were not diminished in asthmatics whether they were on inhaled or oral steroids.2 The primary reason appeared to be a documented decrease in affinity for GR among all asthmatics (the poorest affinity among the oral-steroid dependent group). It is a general pharmacological principle that if affinity is low, more receptors will be expressed by cells – this is equally true in asthma. Therefore receptor number is not the main reason for a lack of response to CS. To understand this, one must appreciate that GR affinity is not static but is altered by disease states and inflammation. This binding affinity changes diurnally3 (yet another reason for more nocturnal symptoms) and over time. The fact that the alteration in affinity is due to inflammation was well demonstrated in a study4 where GR affinity decreased with the onset of the allergen season and, importantly, before the onset of symptoms! In fact, GC are capable of normalising affinity as inflammation is brought under control; the more severe the asthma, the more potent the CS needed to normalise affinity to control symptoms.5

In an attempt to further elucidate the reason for diminished affinity for GR, Leung et al.6 identified a group with steroid-resistant asthma (based on the standard oral corticosteroid challenge) expressing this abnormality. Bronchoalveolar lavage performed in these subjects revealed an increased number of cells expressing positive hybridisation signals for interleukin-2 (IL-2), IL-4 and IL-13 mRNA. Subsequent in vitro studies revealed that altered GR affinity could be induced in peripheral blood mononuclear cells (PBMC) with a combination of IL-2 and IL-4 or IL-13 alone.6,7 Using these data, we developed an in vitro model for steroid resistance to study the mechanisms underlying this altered affinity and the functional consequences thereof.8 Utilising specific inhibitors for the ERK (extracellular regulated kinase) and phosphatidylinositol pathways, Wortmannin and PD098059 respectively, we showed that they were unable to counteract the effect of IL-2. IL-4 on GR affinity suggesting that these signal transduction pathways were not involved. SB203580 on the other hand consistently ameliorated the diminished affinity and number of receptor sites induced by IL-2 and IL-4.9 SB203580 is a specific pyridinyl imidazole inhibitor of p38MAP kinase that prevents activation of its downstream effector MAPK-activating protein kinase 2 (p38MAPK) (p38/MK2/SAPK/PAK) is part of the ras pathway and responds primarily to stressful and inflammatory stimuli, e.g. tumour necrosis factor-alpha (TNFα), IL-1 and lipopolysaccharide (LPS). Potential cellular targets of MAPK include PLA2 and p90/s6 kinase. p38MAPK has been shown to be essential for the mitogenic response of IL-210 and its inhibition predictably normalised affinity. We were also able to show that LPS-stimulated GR-resistant cells generated more GM-CSF and less IL-10 (an ‘anti-inflammatory’ cytokine), suggesting that endogenous mechanisms to control inflammation were impaired.11 This may be a reason why asthmatic inflammation is persistent.

These experiments illustrate therefore that a host of proteins, chaperones and co-regulators, are capable of interacting with GR and are responsible for glucocorticoid resistance or heightened sensitivity in asthma, as well as a number of other medical disorders.

**Synergy between long-acting β-agonists (LABA) and CS**

Clinical studies in adults have unequivocally demonstrated that the best asthma control is achieved with a combination of LABA and CS.12 The development of combination products with these compounds (salmeterol + fluticasone propionate [Seretide: GlaxoSmithKline] and formoterol + budesonide [Symbicort: AstraZeneca]) are set to herald a new era. These products are convenient and improve compliance. Moreover, the clinical data have consistently demonstrated that these compounds are better than the individual components given separately. The benefit probably lies in molecular synergy linked to signal transduction when both molecules are delivered to target cells simultaneously. In one study,13 TNFα-stimulated release of eotaxin (the eosinophil chemo-attractant) from smooth-muscle cells was inhibited most strongly by a combination of salmeterol and fluticasone rather than the single agent alone. What are the reasons for this molecular symbiosis? GC improve β-adrenoceptor number and function by increasing production and protecting against inflammation-induced receptor downregulation and coupling.14,15 In turn, LABA prime the GR for subsequent binding and both increase and accelerate the translocation of GR to the nuclear compartment.16,17 This priming of GR occurs via β-receptor activation of yet another MAP kinase pathway.18

**RECENT DEVELOPMENTS**

**Dissociated steroids**

The undesirable metabolic effects of GC are due to the transactivation modality; thus a steroid with largely transrepressive actions will not have the unwanted effects and will be safer. These are termed dissociated steroids, where a more selective anti-inflammatory profile would represent a major advance and allay fears of CS morbidity. RU 24858 was such a prototype.19

**Soft steroids**

To minimise systemic side-effects, another attribute of an ideal steroid would be one that has high topical selectivity in the Airways but is also inactivated during systemic distribution (not just in the liver). These are soft steroids, currently being researched based on paraoxanase-catalysed breakdown selectivity in plasma.20

**CONCLUSION**

These insights into GR and their interactions have allowed us to better analyse the various aspects of asthmatic inflammation and improve our understanding of the asthma phenotype. The development of new agents, often aimed at attenuating single mediators, is unlikely to have a comprehensive action in curbing inflammation. Corticosteroids do this and will remain the agents of choice for at least the next decade; our challenge is to use them appropriately and safely.
REFERENCES


