



## Review

# Glucocorticoids: As a clinician we use much, we know less

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## ABSTRACT

Glucocorticoids (GC) are frequently used as important drugs in clinical practice of pediatrics. As well as, they have important physiologic functions; they have immune suppressive, anti-inflammatory and anti-allergic effects that they exert on primary and secondary immune cells, tissues and organs. Their therapeutic effects are considered to be mediated by four different mechanisms of action: the classical genomic mechanism of action caused by the cytosolic glucocorticoid receptor (cGR); secondary non-genomic effects which are also initiated by the cGR; membrane-bound glucocorticoid receptor (mGR)-mediated non-genomic effects and finally non-specific, non-genomic effects caused by interactions with cellular membranes. Mechanisms of GR action are transactivation (GR binds to a related element in the promoter region of GC sensitive genes, inducing gene transcription) and transrepression (GR binds to a negative related element in a promoter region of GC-regulated genes that inhibit gene transcription by interfering with the binding of activating transcription factors). The underlying molecular mechanisms for their side effects are complex and frequently partly understood. Recent data suggest that certain side effects are predominantly mediated via transactivation (e.g., diabetes, glaucoma), whereas others are mediated via transrepression (e.g., suppression of the hypothalamic-pituitary-adrenal axis). In this review, we aimed to look at new molecular mechanisms of effects and side effects in relationship with apoptosis and caveolin, intracellular transport and endoplasmic reticulum stress, resistance mechanisms and new drugs developed based on these topics of GCs.

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Glucocorticoids (GC) are frequently used drugs in pediatrics that have immunosuppressive and anti-inflammatory effects. Cortisol, the first GC, was discovered by Kendall and Reichstein in 1937, and then used in a rheumatic disease by Hench in 1948. These pivotal works were awarded the Nobel Prize to them. GCs are among the most important drugs used in clinical practice. Allergic airway diseases, rheumatologic, dermatological and other systemic diseases are in the list of indications (Buttgereitt, 2004). About 10 million new prescriptions are written for oral corticosteroids each year in the United States. Overall the total market size is considered to reach about 10 billion US dollars per year (Schäke et al., 2002).

In this review, we aimed, after a brief clinical knowledge, to look at new molecular mechanisms of effects and side effects, relationship with apoptosis and caveolin, intracellular transport and endoplasmic reticulum stress, resistance mechanisms and new drugs developed based on these topics of GCs.

GCs are 21-carbon steroid hormones. So, they can easily pass through the plasma membrane due to lipophilic structure (Stahn et al., 2007). Their plasma half-lives range between 80 (cortisol) and 270 (dexamethasone) minutes. Except prednisolone, most synthetic GCs are bound predominantly albumin. Mostly, they are metabolized in liver and excreted by the kidneys. GCs mediated their biological effects via the GC receptor (GR) (Schäke et al., 2002; Stahn et al., 2007). Desired clinical effects and undesired adverse reactions were shown on Table 1 and 2. GCs also have important effects on immune system such as neutrophilia, lymphopenia and monocytopenia (Table 1) (Schäke et al., 2002; Stahn et al., 2007). Observed adverse reactions when were used especially at high doses and for long periods is a very long list (Table 2) (Schäke et al., 2002). Because of relation with immune and endothelial cells, GCs have huge clinical (therapeutic or adverse) effects.

**Table 1.** Glucocorticoid effects on primary and secondary immune cells (Aridor and Hannan, 2000).**Monocytes/macrophages (↓ and ↑ symbols mean decreasing or increasing).**

- ↓ Number of circulating cells (↓ myelopoiesis, ↓ release)
- ↓ Expression of MHC class II molecules and Fc receptors
- ↓ Synthesis of pro-inflammatory cytokines (e.g. IL-2, IL-6, TNF $\alpha$ ) and prostaglandins

**T cells**

- ↓ Number of circulating cells (redistribution effects)
- ↓ Production and action of IL-2 (most important)

**Granulocytes**

- ↓ Number of eosinophile and basophiles
- ↑ Number of circulating neutrophils

**Endothelial cells**

- ↓ Vessel permeability
- ↓ Expression of adhesion molecules
- ↓ Production of IL-1 and prostaglandins

**Fibroblasts**

- ↓ Proliferation
- ↓ Production of fibronectin and prostaglandins

**Table 2.** Typical side effects of GCs ordered by organs (Barnes and Adcock, 2009).**Skin**

- Atrophy, striae rubrae distensae
- Delayed wound healing
- Steroid acne, perioral dermatitis
- Erythema, telangiectasia, petechia, hypertrichosis

**Skeleton and muscle**

- Muscle atrophy/myopathy
- Osteoporosis
- Bone necrosis

**Eye**

- Glaucoma
- Cataract

**Central nervous system**

- Disturbances in mood, behavior, memory and cognition
- Steroid psychosis
- Cerebral atrophy

**Electrolytes, metabolism, endocrine system**

- Cushing's syndrome
- Diabetes mellitus
- Adrenal atrophy
- Growth retardation
- Hypogonadism, delayed puberty
- Increased Na<sup>+</sup> retention and K<sup>+</sup> secretion

**Cardiovascular system**

- Hypertension
- Dyslipidemia
- Thrombosis
- Vasculitis

**Gastrointestinal**

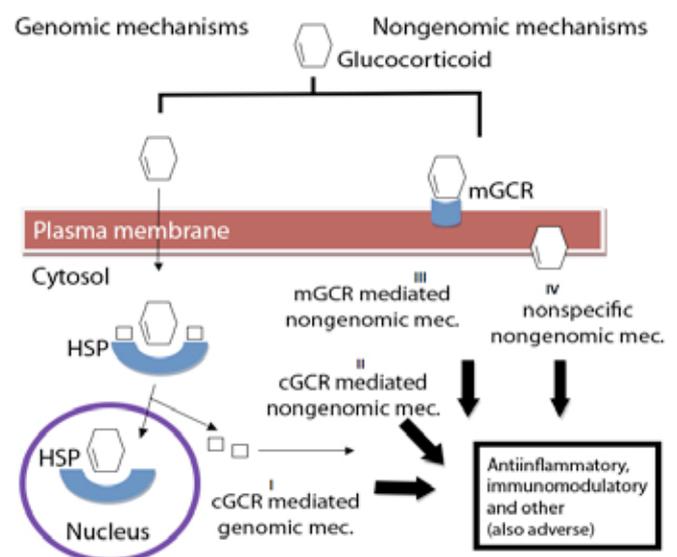
- Peptic ulcer
- Gastrointestinal bleeding
- Pancreatitis

**Mechanism of GR action**

The GR is a ligand-activated transcription factor. This receptor is localized in the cytoplasm as a protein complex with chaperone molecules (a protein that assists the non-covalent folding/unfolding in molecular biology) if its ligand is absent. Two forms of the human GR were described: a steroid binding form (GR $\alpha$ ) and a nonsteroid binding form (GR $\beta$ ). Upon ligand binding, the complex dissociates and the receptor translocates into the nucleus and binds to regulatory elements of GC-responsive gene resulting a modulated gene transcription (Schäke et al., 2002). The specific DNA-binding-sites are called as GC responsive elements (GRE) (Schäke et al., 2002; Stahn et al., 2007). However, there is a controversy concerning the role of GR isoforms. In the peripheral blood of GC-resistant asthmatics, significantly higher numbers of human GR $\beta$  cells were found. This finding suggests a negative function of GR $\beta$  (Stahn et al., 2007). Furthermore, it is thought that apoptosis is mediated through GR $\alpha$  acting on GREs of GC-sensitive genes in T cells and neutrophils (Stahn et al., 2007).

In addition to the GR, there are mineralocorticoid receptors (MR) in the cell. While the GR is widely expressed in most cell types, the expression of the MR is restricted to epithelial cells and nonepithelial cells of brain and heart. Activation of the MR leads to Na<sup>+</sup> retention via an increased activity of epithelial Na<sup>+</sup> channels (Schäke et al., 2002).

Recently, new studies have shown that GC activities can be divided into genomic effects and nongenomic effects. Fig. 1 summarizes these effect mechanisms. It can be seen from Fig. 1 that genomic effects of GCs mediated by GR, non-genomic effects are suggested to be mediated via a membrane bound GR and by interactions with intracellular membranes (Buttgereit et al., 2004).

**Fig. 1.** Summary of genomic and nongenomic Gc action: Lypophytic GCs easily pass through the membrane and bind to the cytoplasmic receptor, which mediate genomic (I) and nongenomic (II) effects. Nongenomic effects are also suggested to be mediated via membrane bound GCR (III) and by interactions with cellular membranes (IV). HSP: Heat shock protein. (Buttgereit, 2004).

### Mechanisms of GC's genomic effects

Three modes of genomic regulation by GR have been described.

Transactivation (i.e. the stimulation of transcription by a specific protein) is mediated via positive GREs and is thought to be responsible for side effects of GCs.

2) Transrepression (i.e. the inhibition of transcription by a specific protein)

a) Directly via negative GREs

b) Indirectly via transcription factors such as activated protein-1 (AP-1), nuclear factor  $\kappa$ B or interferon regulatory factor-3 (IRF-3) related with pro-inflammatory genes leading to anti-inflammatory and immunosuppressive effects.

3) Suppressed transcription of inflammatory genes via negative GREs (Schäke et al., 2002; Buttgereitt et al., 2005; Stahn et al., 2007; Löwenberg et al., 2007).

### Mechanisms of GC's nongenomic effects

Clinically, GCs have rapid immunosuppressive, anti-inflammatory and some anti-allergic effects that couldn't be explained by the classical genomic mechanisms. Three different nongenomic mechanisms proposed to explain these rapid effects:

a) Nongenomic effects which are mediated by the GR (Fig. 1)

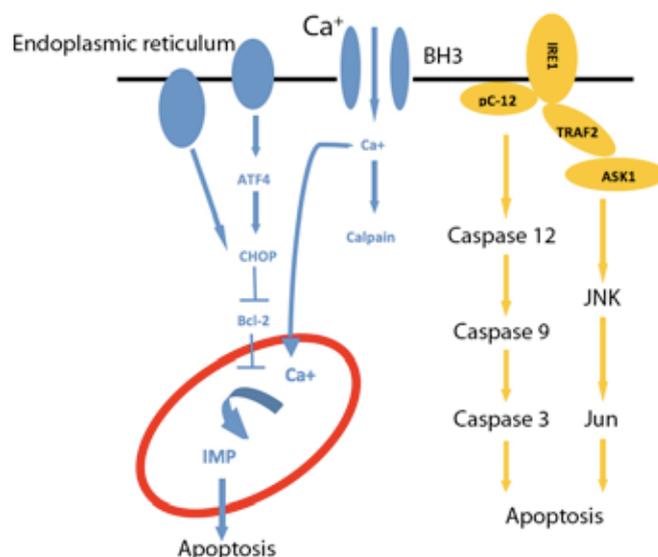
b) Nonspecific interactions of GCs with cellular membranes (Fig. 1)

c) Specific interactions with a membrane-bound GR (Fig. 1-3) (Schäke et al., 2002; Stahn et al., 2007).

Besides, other nongenomic effects including activation of phosphatidylinositol 3-kinase and mitogen activated protein kinase (MAPK) were reported (Matthews et al., 2008). Caveolae and the major component of its caveolin-1 orchestrate these nongenomic effect mechanisms of GCs. Caveolae is a highly ordered plasma membrane microdomain with particular lipid and protein composition and named due to their rigid structure as 'lipid raft' (Matthews et al., 2008).

### Apoptosis and protein trafficking

GCs cause lymphopenia (Table 1) but which mechanism is a longstanding problem (Thompson, 2008). Apoptosis of lymphocytes is mediated by the activation of a variety of cysteine proteases, known as caspases. Simply, there are two way of apoptotic caspase activation; Fas (death receptor)-related extrinsic way and mitochondria-related intrinsic way. Both apoptotic ways can be induced with GCs (Dirks-Naylor and Griffiths, 2009). Recently, a new endoplasmic reticulum (ER)-related apoptosis mechanism was recognized. This development is very important on comprehension of pathologic mechanisms of diseases. Cellular stresses such as oxidative stress, disruption of  $Ca^{2+}$  homeostasis can damage ER function and result in ER stress (Ma et al., 2008). In the ER, proteins fold into their native conformation (Schroder and Kaufman, 2005). Cells protect themselves with a mechanism named

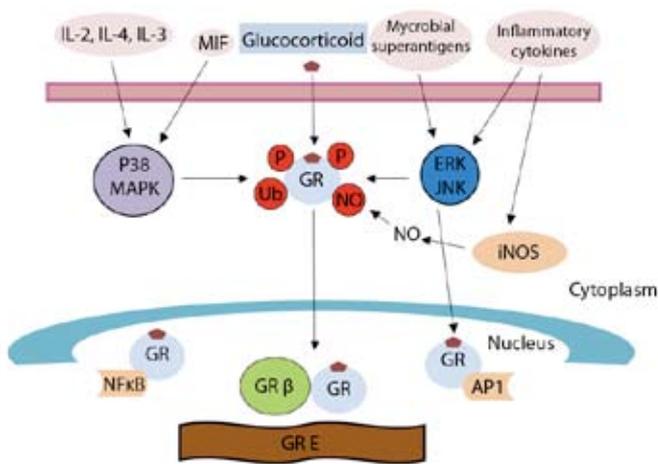


**Fig 2.** Apoptotic pathways regulated by the UPR. IMP: inner mitochondrial membrane potential (Schroder, 2005).

unfolded protein response (UPR) from ER stress. However, prolonged ER stress will impair UPR and trigger an ER stress-specific apoptotic cascade (Ma et al., 2008) (Fig. 2). During the UPR, accumulated unfolded protein is either correctly refolded, or unsuccessfully refolded and degraded by the ubiquitin-proteasome pathway. When the unfolded protein exceeds a threshold, cell goes to apoptosis (Kim et al., 2006). Three specific stress sensors, IRE1, PERK/PEK and ATF6 serve for ER stress (Gass et al., 2008). We do not have enough data whether there are any connections between these stress sensors and GCs yet. For many ER storage diseases, UPR is the biochemical basis, in which folding-incompetent proteins accumulate in the ER (Schroder and Kaufman, 2005). In addition, UPR interact with other cellular signaling cascades that could modulate such as mitogen activated protein kinase (MAPK) and nuclear factor kappa B (NF- $\kappa$ B) that are of nongenomic effect targets of GCs (Rutkowski and Kaufman, 2007).

Apoptosis and GC relation may be also important for tumor cell pathophysiology. Even under extreme ER stress, cancer cells survive due to adaptive processes that are not understood yet (Hersey and Zhang, 2009). However, GCs are able to mediate apoptosis in tumor cells especially in hematological malignancies (Frankfurt and Rosen, 2004).

For a long time, it is known that GCs resolve proteinuria in nephrotic syndrome. But the underlying mechanism remains unknown until a recent study (Fujii et al., 2006). Studies have shown that disruption of protein trafficking underlies various hereditary and autoimmune diseases. This occurs mainly in the ER, which is the central protein folding and transport that are destined to intracellular organelles, plasma membrane or the extracellular space. In minimal change nephrotic syndrome, there is intracellular accumulation of nephrin (a slit diaphragm protein of the glomerul) due to alteration of its intracellular transport may underlie the proteinuria pathophysiology. Fujii et



**Fig 3.** Possible mechanisms of GC resistance. Some kinases can phosphorylate (P) GC receptors (GRs): p38 MAPK, c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK). Nitric oxide (NO) can nitrate GRs. GRs also can be ubiquitinated (ub) which results in degradation of GRs by proteasome (Barnes, 2009).

al. showed that ER stress caused by glucose starvation inhibits the proper intracellular transport of nephrin but GCs improve this transport (Fuji et al., 2006).

Disruption of intracellular protein trafficking is the molecular basis of many hereditary and autoimmune diseases other than proteinuria (Aridor and Hannan, 2009). This development may explain the mysterious therapeutic effect of GCs in many diseases.

### GC resistance in inflammatory diseases

GCs are the most effective anti-inflammatory drugs for many inflammatory and immune system diseases but some patients show a poor or absent response to high doses of

GCs. As above-mentioned, the major action of GCs is to switch off activated inflammatory genes. Why some patients show resistance to GCs remained unclear till the present time. Recently, several molecular mechanisms of GC resistance have been identified due to phosphorylation, ubiquitination and nitrosylation of GRs (Barnes and Adcock, 2009) (Fig. 3).

### New GCs

Many adverse effects of GCs are predominantly caused by transactivation (e.g. diabetes, glaucoma). By contrast, anti-inflammatory effects are mostly mediated by transrepression (such as inhibition of the synthesis of proinflammatory cytokines). Then, new drugs that promote negative regulatory action and reduce positive regulatory action on GR will improve therapeutic index (Schäke, 2007). For this purpose, selective GC-receptor agonists (SEGRA) were developed. Besides, liposomal GCs for effective delivery and nitrosteroids for synergistic effect are other new GC agents (Buttgereit et al., 2005). These new drugs are likely to enter clinical testing soon (Schäke et al, 2007).

### Conclusions

GCs are among the most important but comparatively old drugs used in clinical practice. But some of their effect mechanisms have recently been elucidated. These developments might lead to new therapeutic strategies with GCs in many inflammatory and immune system related human diseases.

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