Pharmacodynamics of glucocorticoids

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ABSTRACT

Exogenous glucocorticoids (GCs) are used as anti-inflammatory and immunosuppressive drugs in the treatment of a wide range of rheumatic and other inflammatory diseases. GCs exert their immunosuppressive, anti-inflammatory and anti-allergic effects on primary and secondary immune cells, tissues and organs via different mechanisms of action in a dose-dependent manner. However, their pleiotropic effects also lead to numerous adverse effects such as unwanted metabolic effects and osteoporosis. The mechanisms of action include the classical genomic mechanism resulting from activation of the cytosolic glucocorticoid receptor (cGCR), non-specific, non-genomic effects caused by interactions with cellular membranes, secondary non-genomic effects initiated by the cGCR and specific interactions with a membrane-bound glucocorticoid receptor (mGCR). Optimised glucocorticoids, such as selective glucocorticoid receptor agonists, are being developed to minimise the adverse effects many patients experience, especially if GCs are given at higher dosages over longer periods of time. Immunostimulatory effects of low concentrations of endogenous glucocorticoids and the influence of pre-receptor metabolism appear of interest for further investigation. The most important approach to optimise the risk-benefit ratio of GCs is to understand in more detail how the molecular mechanisms of genomic and non-genomic GC actions – and their dose-dependency – mediate the clinically wanted benefits but also the known adverse effects.

Introduction

Exogenous glucocorticoids (GCs) are the most commonly used anti-inflammatory and immunosuppressive drugs in the treatment of a wide range of rheumatic and other inflammatory diseases. These substances exert their immunosuppressive, anti-inflammatory and anti-allergic effects on primary and secondary immune cells, tissues and organs via different mechanisms of action in a dose-dependent manner (1-3). The overall physiological effect of endogenous glucocorticoid hormones released by the adrenal cortex is to increase blood glucose concentrations by opposing the action of insulin and increasing glucose production and release from the liver via gluconeogenesis, which is especially important in situations of stress (4).

Hench and his colleagues had noted a correlation between improvement of disease activity in patients with rheumatoid arthritis (RA) and the incidence of jaundice or pregnancy (5). They concluded that there was likely an "anti-rheumatic factor" which is "neither a product of the liver nor a unisexual hormone" (5-7), and this finally led to the application of synthesised cortisone to treatment of RA. Initially, a female patient with severe disease was treated with cortisone in September 1948, with the result of a dramatic improvement of the disease activity (6). Further patients were treated as well and the use of GCs was disseminated over the whole field of rheumatology and medicine.

GCs were used to treat numerous diseases, and the potential of these drugs to induce adverse effects became obvious. Unwanted effects on metabolism, bone tissue, muscles, eyes and skin, and increased susceptibility to infections occur after treatment with higher GC dosages over longer periods of time (3, 8). These adverse events led to synthesis of new GC agents in the 1950-1960s, *e.g.* prednisolone and methylprednisolone exerting stronger anti-inflammatory and immunosuppressive potencies, but weaker mineralocorticoid activities such as sodium/water retention and potassium excretion (9).

In this review we summarise current knowledge of pharmacodynamics of glucocorticoids, including (1) the cellular effects of GCs on immune cells,

(2) the therapeutic effects of GCs, (3) dose-effect-correlations of glucocorticoids with adverse effects, (4) mechanisms of glucocorticoid actions, (5) genomic effects mediated by the cytosolic glucocorticoid receptor (GCR), (6) rapid, non-genomic effects of GCs and (7) optimised conventional GCs and new drugs.

Cellular effects of glucocorticoids on immune cells

Commonly used glucocorticoids like prednisone, prednisolone, methylprednisolone or dexamethasone mediate many anti-inflammatory and immunomodulatory effects on primary and secondary immune cells, tissues and organs (1). On the cellular level, decreases are seen in the number of circulating monocytes/macrophages, their synthesis of pro-inflammatory cytokines and prostaglandins and their expression of MHC class II molecules and Fc receptors. A reduction of circulating T-cells and their production and action of IL-2 (and other cytokines) also is seen. Furthermore, GCs used therapeutically lead to a lower number of eosinophil and basophil granulocytes while the number of circulating neutrophil granulocytes is increased. GC treatment affects endothelial cells through diminished vessel permeability, expression of adhesion molecules, and fibroblast proliferation. Furthermore, production of fibronectin and prostaglandins are decreased by GC (1, 10, 11).

In summary, therapeutically-used glucocorticoids (1):

- Inhibit leukocyte traffic and access of leucocytes to the site of inflammation,
- Interfere with functions of leucocytes, fibroblasts and endothelial cells, and
- Suppress the production and actions of humoral factors involved in the inflammatory process (1).

Therapeutic effects of glucocorticoids

The most important therapeutic effect of GCs is the inhibition of the inflammatory processes, resulting in part from effects on primary and secondary immune cells. The inflammatory process is usually characterised by an up-regulated synthesis of mediators of inflammation such as cytokines or prostaglandins, which finally leads to the typical signs of inflammation: pain, swelling, and loss of function (10).

GCs inhibit nuclear translocation and the function of proinflammatory transcription factors such as activator protein 1 (AP-1) or nuclear factor-κB (NFκB), which are involved in the regulation of the expression of pro-inflammatory genes (12-14). The synthesis of proinflammatory cytokines, *e.g.* interleukin-1 (IL-1), IL-6 and tumour necrosis factor alpha (TNF-α), is dose-dependently reduced as one key result of the so-called "transrepression" (3, 11). These mechanisms may explain in large part retardation of radiological progression in rheumatoid arthritis (RA), as TNF-α and IL-1 stimulate the production of receptor activator of nuclear factor kappa B ligand (RANKL). RANKL supports the generation of mature and active osteoclasts, responsible for bone resorption and erosions in RA (15, 16).

On the other hand, treatment with GCs results in induced synthesis of anti-inflammatory proteins (*e.g.* lipocortin 1, inhibitor of NFκB (IκB)), and also regulator proteins which are important for metabolism. This process is termed "transactivation", and is thought to be responsible for many of the adverse effects of GCs (3, 8).

Correlation between GC dosage, therapeutically desired effects and adverse effects

The most important variable in the likelihood of therapeutically desired and adverse effects of GC is the dosage, modified by the rate of absorption, concentration in target tissues, and affinity of GCs for glucocorticoid receptors (GCRs) (10, 17). GCs, given at high doses and/or over long periods of time are usually clinically very effective, but may induce numerous different adverse effects (8). Undesirable endocrine and metabolic effects include diabetes mellitus, redistribution of body fat, increased body weight, osteoporosis, myopathy, atherosclerosis, and hypertension (8, 18, 19). Other adverse effects include increased risk of infection, depression, cataracts, thinning and ekchymoses of the skin (3, 8, 18, 19).

The main aim of a successful GC therapy is a sufficient treatment of the underlying disease while minimising the dose of the administered GC in order to prevent the occurrence of adverse effects. Therefore, 'low-dose' glucocorticoid therapy, *i.e.* prednisone-equivalent doses of less than 7.5 mg per day, is regarded as optimal maintenance therapy for many patients with rheumatic diseases requiring use of GCs (20). These oral doses result in a saturation of the GCR of less than 40–50% and are known to result in rather mild adverse effects (10, 17, 21). More than 50% receptor saturation is seen with prednisone-equivalent doses of 7.5–30 mg per day. The so-termed 'medium doses' may be initially given in primary chronic rheumatic diseases, but are known to have dose-dependent and considerable adverse effects if used for longer periods of time (2, 18). Therapy with prednisone-equivalent doses of 30–100 mg per day is termed 'high-dose' glucocorticoid therapy, with an almost complete GCR saturation. These doses often result in an successful initial treatment of subacute rheumatic diseases, but cannot be used for long-term therapy because of their high potential for serious adverse effects (18). Likewise, 'very high doses' (prednisone-equivalent of >100 mg per day) of GCs and 'pulse' therapy (prednisone-equivalent of ≥250 mg per day, usually given for 1–5 days) cannot be administered for long-term therapy because of severe adverse effects. Both 'high-dose' and 'very high dose' regimens (i) result in a complete GCR saturation and (ii) produce additional rapid non-genomic GC effects (see below). Therefore, these doses are given in case of potentially life threatening forms of rheumatic diseases, such as systemic lupus erythematosus, myositis, dermatomyositis, vasculitides, and are usually not indicated for most patients with rheumatoid arthritis (20).

Mechanisms of GC action

Both the desirable and unwanted GC effects depend on the structure of the GC

molecule which belongs to the family of steroid hormones and is characterised by a sterol skeleton. A number of empirical studies over many years have established that the 17-hydroxy, 21-carbon steroid configuration is required for glucocorticoid activity through binding to the glucocorticoid receptor (22). Changes in this structure can result in an increase or decrease of specific pharmacodynamic characteristics.

Enhancement of glucocorticoid activity by variations near the C11 atom leads to increased desirable clinical effects, such as in

- Prednisolone, in which a double bond is inserted between C1 and C2,
- Triamcinolone, in which a halogen is included, and
- Methylprednisolone or dexamethasone, both of which are expanded by a methyl or fluoro-group (23).

Adverse effects may be minimised by a reduction of the mineralocorticoid activity of GCs via variations near the C18 atom (*e.g.* methylation or hydroxylation) (23). GCs with an 11-keto instead of an 11-hydroxy group, such as cortisone and prednisone, are prohormones that must be reduced in the liver to their 11-hydroxy configurations (22). Cortisone is converted by hepatic pathways to cortisol, and prednisone is converted to prednisolone, in order to become biologically active (22).

New insights into the mechanisms of GC action suggest that endogenous glucocorticoids are subject to extensive pre-receptor metabolism within target cells or tissues (24). 11β-hydroxysteroid dehydrogenases (11β-HSDs) change the balance between active and inactive glucocorticoids (24, 25). Thus, 11 β-HSD type 1 catalyses the formation of active cortisol from cortisone, whereas 11β-HSD type 2 inactivates active glucocorticoids, these processes being influenced by local inflammation (24, 26, 27). Of note, the solubility, the half-life in the plasma and the affinity to its receptor also influence the pharmacodynamics of glucocorticoids (23).

All effects of glucocorticoids are mediated by genomic and non-genomic mechanisms of action (10, 11, 21, 28-33).

Genomic effects are mediated by the cytosolic GCR (cGCR)

Glucocorticoids are lipophilic molecules, which easily pass through plasma membranes. The glucocorticoid receptor complex, consisting of different proteins (29, 30, 32), is found in the cytoplasm. Since the first detection of the GCR in 1985 (34), a large number of receptor variants has been described, comprising different lengths of the amino-terminus depending on the starting point of translation (35) and different post-translational modifications (such as phosphorylation or sumoylation) that affect the levels of transcriptional activity (36, 37).

After binding of the GC to the glucocorticoid receptor complex with high affinity, the proteins dissociate from the complex (30) and the GC/cGCR complex translocates into the nucleus. There it is able to bind to specific DNA binding-sites (30), resulting in an induced synthesis of anti-inflammatory and regulator proteins ("transactivation", as described above) (2). Furthermore, monomers of the GC/cGCR complex directly or interact indirectly with transcription factors (via "transrepression", as described above) which are involved in the regulation of the expression of pro-inflammatory proteins (*e.g.* IL-1, IL-2, IL-6, TNF-α, Interferon γ (IFN-γ)) (2, 12, 13, 38-40).

Rapid, non-genomic effects of glucocorticoids

Over the years, effects were recognised which occur too quickly to be mediated by the above-mentioned genomic mechanism of GC action. Typically, significant changes on cellular, tissue or organism level become evident after hours or days, but if GCs were given intravenously or intra-articularly at a high dose, rapid clinical effects have been observed. These anti-inflammatory and immunosuppressive effects, also called rapid, non-genomic effects, have been considered to be classifiable into three mechanisms of GC action (31, 41-44):

- Non-specific interactions of glucocorticoids with cellular membranes,
- Non-genomic effects mediated by dissociation of the cGCR multi protein complex, and
- Specific interactions with a membrane-bound GCR (mGCR).

These non-genomic effects are considered to be clinically important at high, very high or pulse doses (prednisone-equivalent >30 mg per day (20). At high dosages, GC concentrations are achieved which can significantly change the physicochemical properties of biological membranes, especially plasma and mitochondrial membranes, resulting in a modification of function and activity of membrane-associated proteins (21, 31). Furthermore, in immune cells, calcium and sodium cycling across the plasma membranes is reduced, which in part may account for immunosuppression and reduction of inflammation (31). ATP production, which is essential to immune cells (*e.g.* for cytokine synthesis, migration, phagocytosis, antigen processing and presentation) also is diminished by inhibiting oxidative phosphorylation and increasing the mitochondrial proton leak (45).

The second class of non-genomic effects is mediated by proteins which dissociate from cGCR-multiprotein complex after binding of GCs to its receptor. Proteins, such as the co-chaperon Src, heat-shock proteins (*e.g.* Hsp90, Hsp70, Hsp56 and Hsp40), immunophilins and kinases of the mitogen-activated protein kinase (MAPK) signalling system are thought to mediate some of the rapid effects of glucocorticoids (42, 46). Glucocorticoids inhibit the release of arachidonic acid, an essential mediator of cell growth and several metabolic/inflammatory reactions. This inhibition of arachidonic acid release can be blocked by the glucocorticoid antagonist RU486 but is insensitive to actinomycin D (42, 45). These observations imply that arachidonic acid release is not dependent on transcription; hence, the cGCR does not only mediate genomic effects, but is also involved in rapid, non-genomic GC actions.

The third possibility of non-genomic GC effects is the specific interaction with membrane-bound glucocorticoid receptors (mGCR), the existence of which was first described in amphib-

Table I. Immunostimulatory effects of glucocorticoids.

Effect	References
Enhancement of IgG synthesis	(66)
Enhancement of nitric oxide (NO) secretion	(67)
Enhancement of pro-inflammatory cytokine secretion, such as IL-1β, TNF-α, IL-6, macrophage inhibitory factor (MIF)	(63, 67-74)
Stimulation of pro-inflammatory molecules, such as glucocorticoid-induced TNF receptor protein (GITR)	(73)
Acceleration of T-cell receptor induced T-cell growth	(75)
Enhancement of T-cell receptor induced CD4 expression	(76)
Stimulation of leukocyte redistribution by – promotion of the survival and proliferation of neutrophils – enhancement of effects of granulocyte macrophage colony-stimulating factor (GM-CSF) – promotion of shedding of L-selectin from neutrophils	(70, 77-81)
Enhancement of the clearance of foreign antigens, toxins, microorganisms, and dead cells by – enhancement of opsonisation – increase of the activity of scavenger systems – stimulation of phagocytotic ability	(63, 68, 70, 78, 82)

ian neuronal membranes and in rodent lymphoma cells (47, 48). Later, this receptor was also detected on human peripheral blood mononuclear cells (21, 49, 50), and first hints on its clinical role were also described (49, 51-53).

Optimised conventional glucocorticoids and new drugs

The above-mentioned mechanisms of glucocorticoid actions suggest possibilities for the development of optimised and/or new glucocorticoids and glucocorticoid receptor ligands, including:

- Conventional GCs can be improved by a targeted delivery via carrier systems (*e.g.* long circulating liposomes); by this route, GCs accumulate directly at the site of inflammation (54, 55),
- GCs can be linked to nitric oxide (NO) which can enhance anti-inflammatory effects of GCs, while it is slowly released from these drugs (56-58),
- Selective glucocorticoid receptor agonists (SEGRAs) cause a receptor conformation preferring GCR/protein interaction rather than GCR/DNA binding, which leads to induced transrepression processes, whereas transactivation remains unchanged (59-61), and
- A new, modified-release prednisone tablet formulation has been developed to prevent the circadian increase of proinflammatory cytokine levels, thereby improving signs and symptoms of rheumatoid arthritis such as the duration of morning stiffness (62).

Immunostimulatory effects of glucocorticoids at very low concentrations also appear of interest for further investigation (Table I) (24). While high concentrations of GCs lead to immunosuppression, concentrations below 10^{-7} M (for cortisol) and below 3 x 10^{-9} M (for dexamethasone), lead to immunostimulation (24, 63). The bidirectional effects of GCs imply that the concentration and timing are decisive in glucocorticoid administration (24). Targeting the pre-receptor metabolism of endogenous glucocorticoids, mediated by 11β-HSDs, may have therapeutic potential, with improvement of both inflammatory processes and metabolic profile (24, 64, 65).

An important general rule to follow for conventional GC use in daily clinical practice is to prescribe "as much as necessary, but as little as possible" (9). Nevertheless, all of these substances – both the conventional and optimised GCs and the novel GCs – may be further developed. The most important approach to optimise the risk-benefit ratio of GCs in human subjects for scientists is to understand the mechanisms of action in more detail, and for clinicians to appreciate important differences between low *versus* high doses of glucocorticoids, administered with optimal recognition of chronobiology (see article in this supplement by Spies).

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