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Glucocorticoids for Human Skin: New Aspects of the Mechanism of Action

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Glucocorticoids · Genomic effects · Glucocorticoid receptor · Non-genomic effects · Cellular signalling · Skin

Abstract
Topical glucocorticoids have always been considered first-line drugs for inflammatory diseases of the skin and bronchial system. Applied systemically, glucocorticoids are used for severe inflammatory and immunological diseases and the inhibition of transplant rejection. Owing to the progress in molecular pharmacology, the knowledge of the mechanism of action has increased during the last years. Besides distinct genomic targets, which are due to the activation of specific cytoplasmatic receptors resulting in the (trans-) activation or (trans-) repression of target genes, there are non-genomic effects on the basis of the interference with membrane-associated receptors as well as with membrane lipids. In fact, various glucocorticoids appear to differ with respect to the relative influence on these targets. Thus, the extended knowledge of glucocorticoid-induced cellular signalling should allow the design and development of even more specifically acting drugs – as it has been obtained with other steroids, e.g. estrogens for osteoporosis prevention.

Introduction

Cortisol, the most potent physiologic glucocorticoid, affects almost all physiologic systems of the organism. Cortisol is synthesised and released from the adrenal cortex under hypothalamic control in a pulsatile and circadian way. Importantly, under stress the adrenals secrete cortisol on top of this rhythm, which facilitates recovery. In case of immune challenge, cortisol protects the organism against damaging inflammatory responses. Due to the multiple targets of action, cortisol and more importantly synthetic derivatives are first-line drugs in the treatment of immunologic and inflammatory diseases, as for example inflammatory bowel disease, arthritis and systemic lupus erythematosus. Moreover, glucocorticoids are effective in the treatment of some neoplastic diseases, e.g. acute lymphatic leukemia of children.

In many inflammatory skin diseases [1] as well as in persistent bronchial asthma [2], topical glucocorticoids are a mainstay of therapy, as unwanted effects are less prominent compared with systemic treatment. With respect to the skin, antiproliferative, apoptotic or antiapoptotic and also vasoconstrictive effects may contribute to the desired anti-inflammatory and immunosuppressive effects, yet may also result in unwanted side effects in other diseases. Moreover, it has been learnt that glucocorticoids do not only differ with respect to potency but also in the benefit/risk ratio [3], which is linked to a selective influence on cytokine synthesis [4, 5]. While this was first

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reported concerning topical glucocorticoid use in dermatology, recently this has also been acknowledged with respect to asthma therapy [6–10]. The understanding of this separation increases since several new aspects of the glucocorticoid signalling pathways were revealed during the last years, expanding our knowledge on the mechanism of drug action. Various aspects of glucocorticoid action have been reviewed previously emphasising comparison of glucocorticoids and other immunomodulators [11], non-genomic glucocorticoid effects [12–14], cross-talk with cytokines and transcription factors [15–18], glucocorticoid-mediated apoptosis [19], receptor isoforms [20] but also effects in asthma [9, 21]. Here, we describe the multifaceted aspects of the mechanism of glucocorticoid action which are currently known and their translation into the therapy of inflammatory skin diseases.

Influence on the Cytokine Network

An imbalance of helper T cells in atopic eczema and asthma results in an increased baseline mediator formation [22]. Consequently, alveolar macrophages of asthma patients and keratinocytes of patients with atopic dermatitis release interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF) at an increased rate [21, 23–25]. By stimulating cytokine receptors on the cell surface, IL-1 and TNF-α induce a cellular signal cascade which activates transcription factors such as signal transducers and activators of transduction (STATs) or the nuclear factor κB (NF-κB) which is released from the complex with its inhibitory protein IκBα. Another relevant pathway of IL-1 and TNF-α is the activation of the mitogen-activated protein (MAP) kinase, and thus the activation of activator protein 1 (AP-1) which also results in the activation of nuclear factor of activated T cells (NFAT) containing AP-1 as an integral part. AP-1 and NF-κB activate response genes by binding to their promotor region, recruitment of co-activators and basal transcription factors. Histone acetylation by activated histone acetyl transferases results in a more relaxed chromatin environment which enables the access of RNA polymerase to the coding region of the target gene. These are genes coding for cytokines (IL-1, IL-2, IL-4, IL-6, IL-13, TNF-α), chemotaxis proteins (e.g., GM-CSF), cellular adhesion molecules, i.e. intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), but also enzymes, i.e. phospholipase A_2, inducible forms of cyclooxygenase (COX-2) and NO synthase (iNOS).

By inducing cellular adhesion molecules, proinflammatory cytokines (e.g., TNFα, IL-1) increase epidermal chemokine formation and T cell localisation. CCL17 and CCL22, chemokines with two highly conserved adjacent cysteine residues formed by IL-4 and IL-13 stimulation, recruit T cells to skin and lung. Keratinocytes from patients with atopic dermatitis express also other Th attractant chemokines such as RANTES, MCP-1 and – most importantly – CCL27 more abundantly [23, 24].

Most importantly, there is an intense cross-talk of the various signalling pathways described, and maximum activation of a cell means the activation of more than a single pathway. Moreover, enzymes from the MAP kinase pathway may also interact with the NF-κB cascade [15, 16].

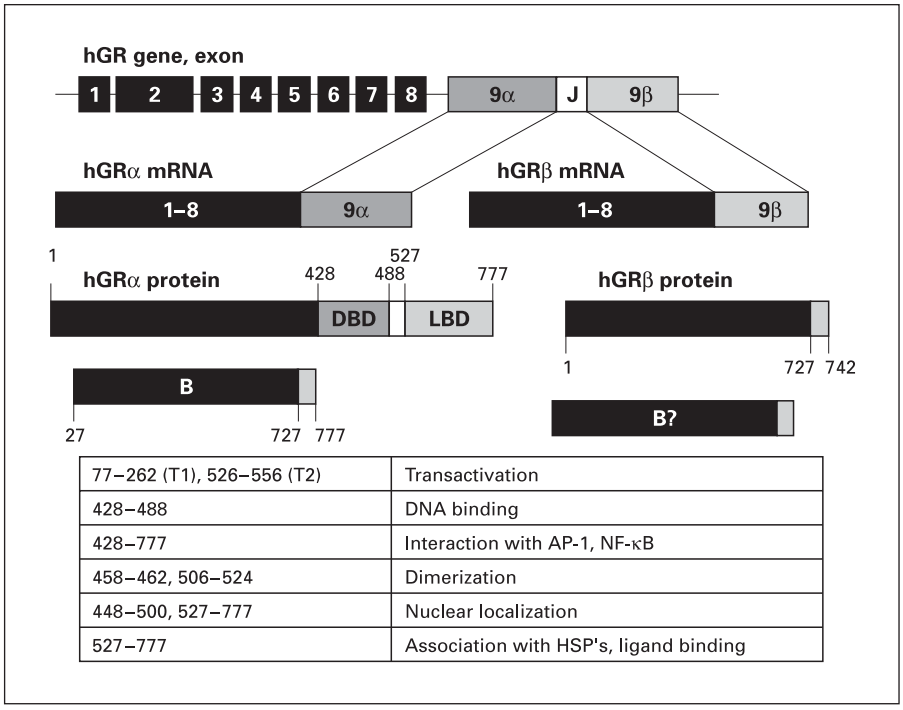
By inhibiting the formation of inflammatory cytokines, glucocorticoids interfere with the activation of a variety of immunologic cells. Glucocorticoids inhibit the activation of dendritic cells which normally stimulate Th2 cells and in the lung also B cells. Moreover, eosinophil and T cell apoptosis increases. Yet, glucocorticoids also influence other cells such as keratinocytes, fibroblasts and alveolar cells. Besides genomic effects, non-genomic targets appear to contribute to glucocorticoid effects.

Genomic Glucocorticoid Effects

Receptor Structure

In general, steroid hormones induce their effects by binding to intracellular receptors (glucocorticoid receptor, GR or hGRα; mineralocorticoid receptor, MR; progesterone receptor, PR; androgen receptor, AR; estrogen receptor, ER; fig. 1) which are closely interrelated and form a superfamily together with the receptors for thyroid hormone, retinoids, vitamin D but also the peroxisome proliferator activated receptor. Today, various isoforms of these receptors are known. These proteins are characterised by an N-terminal domain activating gene expression, a central DNA-binding moiety and a C-terminal ligand-binding domain.

The highly conserved central DNA-binding domain of about 70 residues contains two loops which are stabilised by four cysteine residues each complexing to zinc ions (zinc fingers). The N-terminal zinc finger discriminates between the different response elements and therefore is important for the selectivity of glucocorticoid effects. The DNA-binding domain is flanked by an N-terminal transactivating moiety (τ1) which after receptor binding enhances the transcription of target genes. The ligand-bind-



77–262 (T1), 526–556 (T2)	Transactivation
428–488	DNA binding
428–777	Interaction with AP-1, NF-κB
458–462, 506–524	Dimerization
448–500, 527–777	Nuclear localization
527–777	Association with HSP's, ligand binding

Fig. 1. Human glucocorticoid receptor gene, mRNA splice variants and receptor proteins (hGRα and hGRβ); modified from [20].

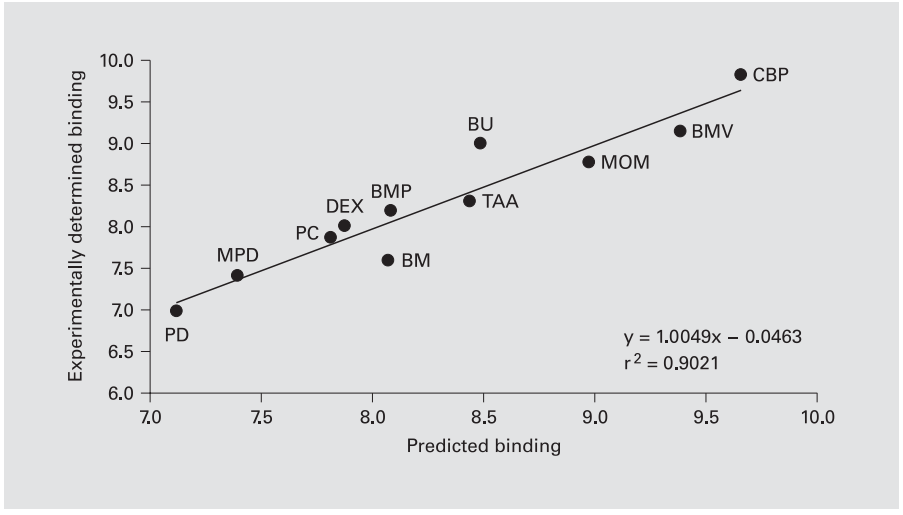


Fig. 2. Glucocorticoid receptor binding as determined experimentally [30, 81]. BM = Betamethasone; BMP = betamethasone dipropionate; BMV = betamethasone 17-valerate; BU = budesonide; CBP = clobetasol 17-propionate; DEX = dexamethasone; MOM = mometasone; MPD = methylprednisolone; PC = prednicarbate; PD = prednisolone; TAA = triamcinolone acetonide.

ing domain is built up from 12 α-helices, the segment τ2 of the liganded receptor, which is part of helix 12, favours dimerisation and enhances transactivation but also interactions with co-activators [26]. The closest homology is found within the group of the 3-oxo-steroid receptors which besides GR are the MR, PR and AR. The ligand-binding domains contain a central arginine and a glutamine complexing the 3-keto group, while it is glutaminic acid and arginine of ER which interact with the phenolic group of the estrogens [26, 27].

Recently, the precise structure of the ligand-binding domain of GR was revealed by the crystallisation of this domain [28] and independently by molecular modeling starting from the well-known ligand-binding domain of PR [29, 30]. The C-terminal 12 helices of the GR fold into a three-layer sandwich which embeds the hydropho-

Table 1. Glucocorticoid receptor binding predicted from quantitative structure activity calculations and experimentally determined [30, 81, 82], IC_{50} values for genomic and non-genomic effects in A549 cells [7] and relative activity in rheumatic diseases [13]

Glucocorticoid	Binding		Genomic effects			Non-genomic effects	
	calculated	determined	growth inhibition	COX-2	PGE_2	AA/ PLA_2	antirheumatic activity
Dexamethasone	7.87	8.02 8.03	8.30	7.00	7.70	7.70	1.2
Methylprednisolone	7.39	7.43	8.30	NS	8.00	8.00	1.0
Fluticasone	NR	9.3	<12.00	9.30	9.30	NS	ND
Mometasone	8.97	8.79 9.12	<12.00	10.00	11.00	NS	ND
Budesonide	8.48[1]	9.00	10.30	9.00	9.00	NS	ND
Beclomethasone dipropionate	8.70[1]	ND	12	9.12	9.12	NS	ND
Beclomethasone	7.86[1]	ND	9.60	6.70	7.70	7.70	ND
Prednylidene	7.52[1]	ND	ND	ND	ND	ND	3.0
Prednisolone	7.12	6.99	9.00	7.30	7.30	NS	0.4
Betamethasone	8.07	7.60	ND	ND	ND	ND	0.2
Betamethasone 17-valerate	9.38	9.15	ND	ND	ND	ND	ND
Betamethasone dipropionate	8.08	8.20	ND	ND	ND	ND	ND
Prednicarbate	7.81	7.89	ND	ND	ND	ND	ND
Clobetasol 17-propionate	9.65	9.82	ND	ND	ND	ND	ND
Hydrocortisone	7.20[1]	ND	8.00	7.12	7.12	7.12	ND
Triamcinolone acetonide	8.44[1]	8.31	7.60	6.70	6.70	6.70	ND

ND = Not determined; NR = reliable results not to be expected; NS = not significant.
[1] Recalculated as described by [30].

bic pocket for the ligand. In contrast to other nuclear receptors, there is a rather wide side pocket which allows access of many steroid esters favoured for the topical treatment of inflammatory skin diseases and bronchial asthma. Results of ligand docking experiments to predict glucocorticoid activity conformed to steroid binding determined experimentally. As depicted in figure 2, there is a close correlation of GR binding predicted by quantitative structure activity calculations and the binding data determined experimentally. Extending the set of 17 steroids [30] by another seven (table 1) the only exception is fluticasone propionate, calculation of binding data is excluded since this agent is the only carbothiolate drug.

Non-stimulated GRs form a complex with various receptor-associated proteins (RAPs) which mask the ligand-binding domain. RAPs are chaperones (heat shock proteins hsp90 and hsp70) which ensure correct folding of the receptor protein and co-chaperones such as the immunophilin FKB51 [18], a FK506 (tacrolimus)-binding protein.

Receptor Activation, Response Elements, Transactivation and Transrepression

Glucocorticoid binding induces hyperphosphorylation, exchange of immunophilins (FKB52 replaces FKB51) and recruitment of the transport protein dynein which allows nuclear translocation of the receptor. In the nucleus, ligand-activated GRs release hsp90 and change structure [18] which allows the formation of a unique homodimer characterised by a β-sheet made up from the dimerisation domains of both monomers [28]. GR dimers as transcription factors bind to specific DNA-binding elements (glucocorticoid response elements; GREs) and influence the expression of target genes which harbour the respective response elements in their promotor region (simple GRE, transactivation; fig. 3a). Simple GREs are imperfect palindromic 15-base-pair sequences which are recognised by the zinc fingers of the DNA-binding domains of activated receptors. Gene inducibility increases with the numbers of response elements in the vicinity of the polymerase-binding moiety. Composite GREs allow additional binding for non-receptor factors

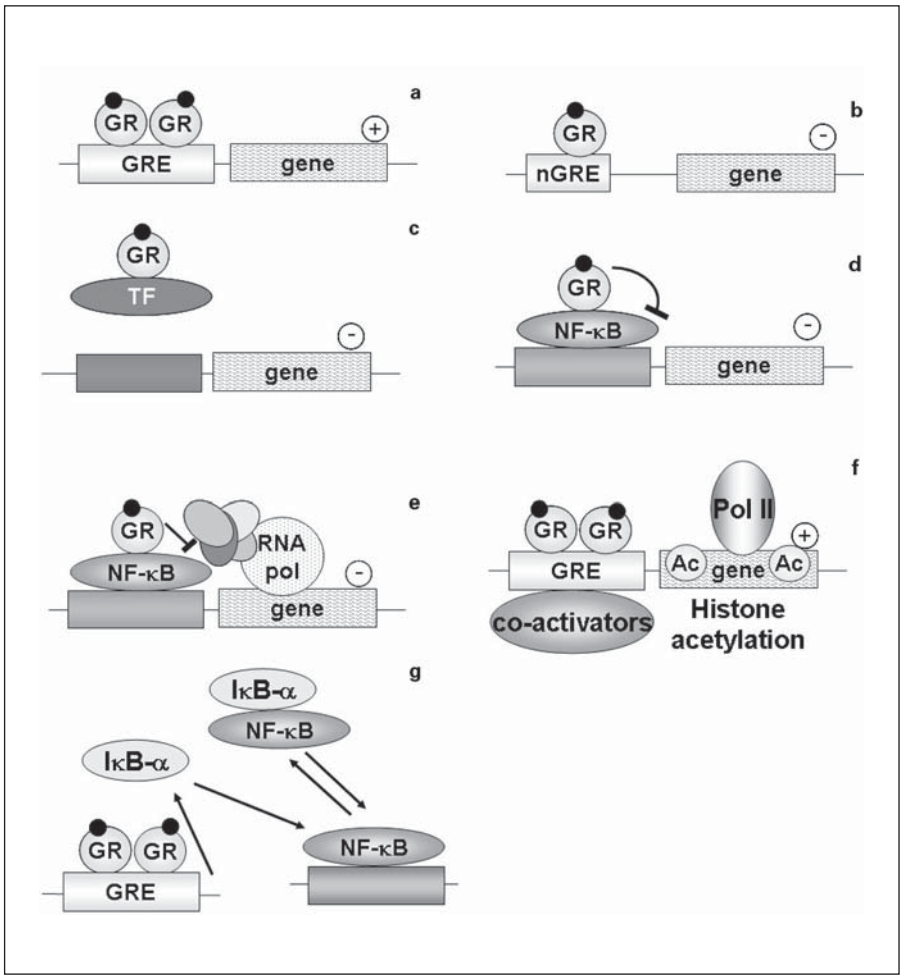


Fig. 3. Models describing glucocorticoid interactions with DNA and transcription factors (modified from [18]; for details see text).

[18]. Glucocorticoid-induced transactivation is seen, e.g. with genes related to gluconeogenesis, arterial blood pressure and intraocular tension which harbour GREs as does the gene encoding the β_2-adrenoceptor.

Besides inducing gene expression by binding of a homodimer to a GRE, glucocorticoids may also inhibit gene expression either by the binding of liganded monomers to a promotor containing negative GREs (nGREs; fig. 3b) or by the sequestration or inhibition of basal transcription factors (fig. 3c–e). nGREs differ in structure from classic GREs and were first described for the proopiomelanocortic gene [21] and then for others, e.g. the IL-1β gene [18]. In epidermal cells, the synthesis of basal-cell-specific (K5, K14) and disease-associated keratins (K6, K16, K17) is under the control of four nGREs [31]. Another form of inhibited gene expression has been known even longer. This is due to the complex formation of liganded GR monomers and the basal transcription factors described above which then become inactivated either by sequestration (fig. 3c), masking of transactivation domains (fig. 3d) or steric hindrance of the basal transcription machinery (fig. 3e). Residues of the GR DNA and ligand-binding domains interact with AP-1, the DNA-binding domain is involved in the complex formation with NF-κB.

Helix 12 of the activated GR allows co-activator and/or co-repressor interaction [28], including the steroid receptor co-activator (SRC)-1 and the cAMP-responsive element-binding protein (CREB)-binding protein (CBP). Increasing histone acetylation co-activators (fig. 3f) weaken histone-chromatine interactions which enables polymerase to initiate transcription, while the opposite holds true with histone deacetylase. By binding to co-factors, glucocorticoids reverse these effects on gene expression. In addition, glucocorticoids may influence gene expression by histone methylation and phosphorylation (for review, see [9]).

Table 2. Glucocorticoid activation and suppression of genes relevant for the efficacy in inflammatory skin diseases and skin atrophy induction (from [18])

Inhibition of	Gene	Transcription factor
Cytokines	IL-2	NF-AT, AP-1, NF-κB
	IL-6	NF-κB, AP-1
	TNF-α GM-CSF, IL-1β	NF-κB
	IFN-γ	AP-1
Enzymes	iNOS, COX-2	NF-κB
	collagenase	AP-1
Adhesion molecules	ICAM-1, E-selectin, VCAM-1	NF-κB
Skin proliferation	keratin	GR monomer (nGRE)
	collagenase	AP-1

Another genomic effect includes the increase in glucocorticoid-inducible leucin zipper and thus AP-1 [18] and NF-κB formation in macrophages [32]. Inhibition of lymphocyte and monocyte activation by the NF-κB pathway (fig. 3g) is due to an enhanced transcription of the IκBα gene [21, 33, 34] which, however, is not the case with murine fibroblasts, endothelial cell lines [35] and human keratinocytes [5]. Table 2 summarises signalling pathways which are influenced by glucocorticoids.

Moreover, glucocorticoids interfere with the formation of inflammatory proteins also at the post-transcriptional level by mRNA destabilisation as described for, e.g., TNF-α, IL-1, IL-2, IL-6, COX-2 and iNOS [18].

Glucocorticoid Antagonists

As with other nuclear receptors, GR antagonists have been developed. Most frequently used for in vitro and animal experiments is RU486 (mifepristone) which inhibits dexamethasone-induced transactivation, while it acts as a partial agonist with respect to AP-1 and NF-κB suppression [36–38]. RU486 also induces immunophilin exchange, dynein recruitment and nuclear translocation [39]. In man, RU486 suppresses the symptoms and signs of hypercortisolism and dexamethasone-induced pituitary inhibition and skin blanching [40]. Most interestingly, the glucocorticoid antagonist ZK98299 acts in a more specific manner as the liganded GR does not interfere with NF-κB activity [36, 37]. This distinct interaction offers the horizon to more selective GR ligands.

Receptor Isoforms

Several isoforms of the human GR can be differentiated. Only in rare cases, however, point mutations or splice variants may result in diseases, e.g. rare forms of the glucocorticoid resistance syndrome [20]. The most important form is the broadly distributed α-form (hGRα; fig. 1) containing 777 amino acids which is expressed in high amounts in almost all cell types including keratinocytes and fibroblasts [41], endothelial cells of blood vessels and the airway epithelium as well as the epithelium of bronchial vessels [21].

Breslin et al. [42] identified two more exons upstream of exon 1, which can translate into exon 1 of the hGR protein; these are therefore named exons 1A and 1B, while exon 1, known for a longer period now, is called exon 1C. Moreover, alternative translation initiation of exon 1 (1C) results in the formation of a B isoform of hGRα which consists of 751 residues [43]. Currently, the tissue distribution and possible differences in function of the isoforms differing in the transactivating domain await to be discovered.

Another broadly distributed isoform, named hGRβ, is generated from a splice variant of exon 9 of the GR gene and is found in human tissues (e.g., lung, thymus, lymphocytes, brain, heart, muscle), though it is present at a lower rate as compared with hGRα (1:40–2,800) [20]. Both isoforms are identical until amino acid 727, then 15 different residues follow in hGRβ. Due to the truncated C-terminal domain, hGRβ does not bind ligands any more and is transcriptionally inactive itself. Early investigations suggested a negative effect of hGRβ on hGRα effects [44, 45]. This, however, may be related to some tissues or cell states only [20]. A protective effect of hGRβ against dexamethasone induced apoptosis of human neutrophils [46] and a correlation of hGRβ expression, and glucocorticoid resistance were described [47–49]. Yet, the relevance of hGRβ for asthma is still under debate [9, 49, 50]. Following cotransfection of hGRα and hGRβ genes, transactivation declined by 40% in COS-7 cells and to a lesser extent in the lung carcinoma cell line A549. Most interestingly, overexpression of either isoform inhibited transrepression – even in the absence of dexamethasone stimulation which might be due to complex formation of even the non-liganded GR and transcription factors [50].

hGRα mRNA also clearly dominates in primary cultures of human keratinocytes (328-fold) and fibroblasts (710-fold) over hGRβ mRNA. Increasing hGRβ over hGRα fivefold by cotransfection of COS-7 cells did not reduce the binding of glucocorticoids and RU486 significantly [41]. In skin biopsies, hGRβ is upregulated during tuberculin response [48]. All these ratios, however, have to be interpreted with caution since mRNA ratios must not translate 1:1 to the protein level and the quantification of the protein is related to antibody specificity.

Another truncated splice variant (GR-δ; exon 8) detected in tumour cells or the rather ubiquitously expressed hGR-γ do not seem to be of major clinical relevance [20]. Yet, this may be different with two other recently described polymorphisms. An S651F mutation of hGRα was identified in immortalised lymphocytes isolated from atopic Japanese, resulting in the formation of a less stable and therefore less active GR protein. Stability of mRNA was even more reduced if adenine was inserted into position 2314 of hGRα gene. Detected with lymphocytes taken from patients suffering from lupus nephritis, this polymorphism may reduce the efficacy of glucocorticoid therapy [51].

Concerning tissue specificity, another GR polymorphism deserves to be mentioned, which is due to alternative splicing of human GR gene by two forms of the DNA restriction enzyme BclI. While this does not change the coding region of the GR gene, it may influence the regulating region [18]. Most interestingly, the large variant of the restriction enzyme was linked to increased sensitivity to budesonide-induced skin blanching, while dexamethasone more efficiently inhibited lysozyme release from leukocytes from subjects with the small allele [52]. Once more, this hints at tissue-specific effects of various glucocorticoids.

In the context of GR isoforms, also the GR^{dim} mouse has to be mentioned, which is transfected with a GR isoform unable for dimer formation and thus transactivation, yet inducing transrepression as the wild type does. Therefore, in contrast to the GR knockout mice, the GR^{dim} mouse grows until adulthood [53].

Modulation of Responsiveness

Besides GR isoforms and the numbers and locations of GREs in the promotor, post-translational processes regulate glucocorticoid responsiveness. Hyperphosphorylation of serins in the τ1 region of the liganded GR enhances the response to steroids by favouring ligand binding, RAP binding and nuclear trafficking, while GR dephosphorylation reduces transactivation. Moreover, glucocorticoid response is influenced by the cross-talk of nuclear receptor signalling pathways, since the liganded GR does not only form a homodimer, but also heterodimers with the other nuclear receptors and can compete with those for co-factors [18]. Also receptor-associated immunophilins may induce a cross-talk with other signalling cascades as to be derived from studies in synovial fibroblasts [54] and the lung adenocarcinoma-derived A549 cell [8] stimulated with dexamethasone and FK506.

Recently, tyrosine nitration has been described as a mechanism to enhance GR hsp90 dissociation and anti-inflammatory activity. Nitration, including the important Tyr735 [29], was induced by a GR ligand carrying a nitration domain [55]. This observation may stimulate the search for new and possibly more specific drugs.

Glucocorticoid Resistance

Whether hGRβ is of relevance with respect to glucocorticoid resistance which occurs, e.g. in up to 10% of asthmatic subjects but also in other chronic inflammatory diseases, currently remains open. Alternatively, a reduced number of activated GR within the nucleus, a lack of interaction with the basal transcription process, or a hyperactive MAP kinase pathway may be the reason for this steroid resistance [15]. It can only be speculated whether one of these mechanisms may be of relevance also in glucocorticoid tachyphylaxia, which was a subject of major concern in the early days of topical glucocorticoid treatment of skin disease [56].

Non-Genomic Glucocorticoid Effects

While it takes at least 30 min for the genomic effects to become obvious in a cell culture experiment, it takes only seconds or a few minutes to detect non-genomic effects. As with other steroids, non-genomic targets were identified also with glucocorticoids [12–14]. Membrane-associated receptors appear to be a specific isoform or a modification of the classical GR. Steroid effects on membrane-associated receptors encompass the activation of MAP kinase, adenylate cyclase and protein kinase C [18]. Glucocorticoids inhibit the recruitment of signalling factors of activated epithelial growth factor receptors and thus phospholipase A_2 and arachidonic acid release [57]. Investigations of various glucocorticoids in A549 cells allowed to separate three types of glucocorticoids (table 1; fig. 3). The conventional steroids dexamethasone, hydrocortisone, triamcinolone acetonide and beclomethasone induced both genomic (COX-2 expression, prostaglandin

E release and inhibition of cell growth) and non-genomic effects (phospholipase A_2 activation and arachidonic acid release). The latter was proven by its sensitivity to the SRC-1 inhibitor PP2 and insensitivity to the translocation inhibitor geldanamycin. In contrast, methylprednisolone exhibited only the non-genomic effect, while the newly developed steroids fluticasone 17-propionate, mometasone and budesonide but also beclomethasone dipropionate did not induce phospholipase A_2 activity, yet selectively inhibited COX-2 expression, prostaglandin E release, and cell growth [7]. In fact, these steroids are reported to induce less side effects in asthma therapy as compared with conventional steroids. These data clearly indicate that a separation of glucocorticoid effects is possible. Since RU486 inhibited genomic and non-genomic effects, the latter should be induced by the stimulation of membrane-associated GR.

If applied at very high concentrations (prednisolone >200 mg), which are used for, e.g., shock therapy, glucocorticoids can also induce non-specific non-genomic effects, possibly due to a physical dissolution of the steroid molecule within the cell membrane. This results in a partial uncoupling of oxidative phosphorylation, mitochondrial proton leak and a decrease of cellular entry of calcium ions which may explain that high glucocorticoid doses are generally needed for acute exacerbations of immunological diseases. Relative glucocorticoid potencies for non-specific non-genomic effects [58] clearly differ from potency for genomic effects (table 1). With respect to the topical treatment of skin disease or bronchial asthma, local drug concentrations high enough to induce non-specific non-genomic drug actions currently cannot be excluded.

Glucocorticoid Effects on the Skin

Despite of the recent introduction of calcineurin inhibitors into the topical treatment of eczema, glucocorticoids still are first-line drugs, especially with respect to the acute treatment of the more severe forms [1]. Yet, topical glucocorticoids are also indicated for other inflammatory skin diseases, as for example psoriasis. This is due to the inhibition of the formation of inflammatory proteins released from keratinocytes, fibroblasts and leukocytes infiltrating the skin. Unwanted effects most feared are skin thinning which is due to the inhibition of keratin synthesis of epidermal cells [31] and, more importantly, to an impaired proliferation of fibroblasts [5, 59, 60] and disturbed collagen metabolism in the dermis. In the rat, dexamethasone induced a drastical reduction of the fibril type III collagen and – less – type I collagen. Moreover, this drug decreased collagenases which belong to the matrix metalloproteinases (MMP) and tissue inhibitors of the metalloproteinases TIMP-1 and TIMP-2 [61]. In fact, collagenase (MMP-13) produced by basal keratinocytes is under AP-1 control and the formation is downregulated by dexamethasone treatment of both wild-type and the GR^{dim} mice [33]. Since type I and type III collagens primarily determine the tensile strength of the skin [62], this deterioration explains well striae formation which occurs in hypercortisolism and may follow long-term topical treatment with glucocorticoids [63]. Moreover, the effect on K5 and K14 explains epidermal thinning during long-term glucocorticoid treatment, inhibition of K6 and K16 the interference with wound healing. The additional effect on keratin synthesis via AP-1 inhibition may increase glucocorticoid effects, since besides nGREs these keratin gene promotors also contain an AP-1 response site [31].

Glucocorticoids induce cytostatic and cytotoxic effects in a cell-specific manner [62]. While apoptosis induction is a well-known effect of glucocorticoids interfering with NF-κB in lymphocytes, and the inhibition of immune cells should contribute to its immunosuppressive and anti-inflammatory effects in eczema and psoriasis, skin thinning following glucocorticoids is not due to an increased apoptotic rate. In fact, despite an inhibition of NF-κB activity, dexamethasone protected fibroblasts against the TNFα, UV irradiation or ceramide-induced apoptosis by stimulating sphingosine 1-phosphate (S1P) formation. While NF-κB inhibitors did not abolish the antiapoptotic effect, the sphingosine kinase inhibitor N,N-dimethylsphingosine did so. This effect is cell specific as it was not seen with keratinocytes [64]. Although we previously demonstrated that vitamin D_3 and the transforming growth factor-β stimulate fibroblast proliferation via an increased sphingosine phosphorylation [65, 66] and S1P then activates Smad proteins [67], the S1P-induced proliferation is obviously overrun by the antiproliferative effect of glucocorticoids due to the inhibition of IL-1α and IL-6 release [5].

Glucocorticoids: Benefit/Risk Ratio in Eczema Therapy

Since target diseases for glucocorticoid treatment are of chronic or relapsing nature, drugs efficacious and safe in the long-run are of highest relevance. The unraveling

of signalling processes offers new perspectives for safer therapeutic strategies. Antiproliferative effects of topical glucocorticoids, however, do not parallel steroid potency in man [68, 69] or cell culture [5]. When looking into the molecular reason for the partial separation of wanted and unwanted effects obtained so far, one has to take the multi-faceted mechanism of action into account.

Comparing hGRα binding of 17 topical glucocorticoids widely varying in potency (prednisolone to clobetasole 17-propionate) did not allow to detect an influence of receptor affinity on the benefit/risk ratio. In fact, binding of less atrophogenic steroids fitted well into the spectrum of the more toxic agents [30]. Moreover, calculated receptor binding of six glucocorticoids studied for their influence on A549 cells are well in accordance with previous data (table 1, fig. 2). Yet as with asthma, the differences in the benefit/risk ratio in eczema may result from differences in transrepression, transactivation and specific non-genomic effects [7] undistinguished by receptor binding. Interestingly, low glucocorticoid concentrations appear to induce transrepression more potently than transactivation [9]. The influence on NF-κB transrepression, however, did not reflect the potential in skin diseases. Indeed, clobetasol propionate activity only slightly exceeded the hydrocortisone effect [38]. Differentiating steroids with a favourable effect on transrepression in vitro [70, 71], however, failed in the animal experiment [72]. Therefore, additional targets appear of relevance.

Mometasone and the relatively equipotent fluticasone propionate [73] both belong to the group of glucocorticoids with an improved benefit/risk ratio [69]. At lower concentrations, they bind preferentially to GR monomers, and induce dimer formation only at higher concentrations [6]. In addition, a separation of budesonide and dexamethasone effects has been described in subjects expressing different GR isoforms [52].

Therefore, the specific interaction of a glucocorticoid with the GR may influence the structure of the receptor surface and thus its interaction with other proteins and GREs. In fact, recently differing requirements of GR targets for τ1 and τ2 structures were reported [62], and full and partial GR agonists differing in D ring structure induce different receptor conformational changes [29]. Interestingly, prednicarbate, which proved less atrophogenic than betamethasone 17-valerate and also less than mometasone [69], turned out as a partial agonist with the N564A mutant of hGRα [30].

Since glucocorticoid effects depend on the cell type, cell culture experiments, especially if performed in cell lines or using protein overexpression, may not exactly reflect glucocorticoid effects in vivo. In fact, glucocorticoids less atrophogenic to the skin (mometasone furoate, fluticasone propionate) strongly inhibit the proliferation of A549 cells (table 1) [7]. Detecting skin-specific effects asks for studies in vivo or at least in primary skin cell cultures. Doing so, however, does not eliminate the 'time' factor, since cell culture experiments are run for some hours, while the treatment of skin diseases lasts for days or even weeks which allows for adaptative processes. Therefore, it is to be expected that in vivo experiments will improve further our understanding of glucocorticoid-related differences in wanted and unwanted effects.

Future Developments

Glucocorticoid research is an ongoing process for more than 50 years, and its intensity is currently increasing. New congeners of a somewhat improved benefit/risk ratio have been developed [63], more selective ones are to be expected. Current research aims at designing drugs inducing selective transrepression [71, 72] or enhancing binding by nitrotyrosine formation [55]. Increasing target selectivity at the molecular level, however, may bear the risk of a major loss in efficacy.

An alternative for a safer eczema therapy therefore encompasses drug carrier systems for epidermal glucocorticoid targeting [74, 75]. Moreover, combined treatment with tretinoin should reduce skin atrophy [76, 77], and ointment preparations containing a glucocorticoid plus a vitamin D analogue may improve psoriasis therapy by reducing side effects [78]. Studies in rheumatoid synovial [54] and murine skin fibroblasts [79] also indicate a synergistic effect of tacrolimus and low-dose dexamethasone which may translate into clinical improvement. Due to the still measurable potential for skin atrophy of glucocorticoids and the lower potency and the risk of photocarcinogenesis with tacrolimus and pimecrolimus [80], a combination therapy that is safe and effective in long-term treatment of severe cases would be welcome.

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