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REVIEW

Glucocorticoids in the Treatment of Rheumatic Diseases

An Update on the Mechanisms of Action

Frank Buttgereit,[1] Rainer H. Straub,[2] Martin Wehling,[3] and Gerd-Rüdiger Burmester[1]

Introduction

Six years after we published our first article on the mechanisms of action of glucocorticoids (GCs) (1), there is already a need for an update. GCs are superior to many drugs in terms of the number of patients treated, the variety of potential uses, and the experience with treatment in humans. They still represent the most important and most frequently used class of antiinflammatory drugs, and their therapeutic use has risen continuously in recent years (2). About 10 million new prescriptions for oral GCs are written each year in the US alone (2). They are the silent companions of rheumatologists, and it is impossible to imagine therapy—especially oral therapy, but intravenous and intraarticular as well—without them. From community survey data, the frequency of oral GC use has been estimated to be 0.5% of the general population and 1.75% of women over the age of 55 years (3,4). Between 56% and 68% of patients with rheumatoid arthritis are treated more or less continuously with GCs (5–8). GCs are relatively inexpensive drugs, but it is the sheer volume used that is significant overall; the total market size is believed to be about 10 billion US dollars per year (2).

Our understanding of the actions of GCs has also greatly increased in the last few years. In this review, we report on recent insights relating to 1) signaling, transcription processes, and gene expression as induced, inhibited, and/or modified by the interaction of GCs with their cytosolic receptors, 2) relationships between dosages and plasma levels, 3) membrane-bound GC receptors (GCRs), and 4) new (glucocorticoid) drugs on the horizon. These data support the modular concept we proposed in 1998 (1), and so this update follows the same structure.

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[1]Frank Buttgereit, MD, Gerd-Rüdiger Burmester, MD: Charité University Hospital, Berlin, Germany; [2]Rainer H. Straub, MD: University Hospital, Regensburg, Germany; [3]Martin Wehling, MD: Mannheim Hospital, Ruprecht Karl University, Heidelberg, Germany.

Address correspondence and reprint requests to Frank Buttgereit, MD, Department of Rheumatology and Clinical Immunology, Charité University Hospital, Schumannstrasse 20/21, 10117 Berlin, Germany. E-mail: frank.buttgereit@charite.de.

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Clinical background: different dosages and dosing regimens have distinct therapeutically relevant effects

The basis for the use of different dosages of GCs for different clinical conditions is essentially empirical, since the evidence to support preferences in specific clinical settings is markedly scarce (9). It is clear, however, that the dosages are increasing with increasing clinical activity and severity of the disease under treatment. The rationale for this (mostly successful) clinical decision is as follows. First, higher dosages increase GCR saturation in a dose-dependent manner (Table 1), which intensifies the therapeutically relevant *genomic* actions discussed below. Second, it is assumed that with increasing dosages, additional and qualitatively different *nonspecific nongenomic* actions of GCs come increasingly into play.

Current knowledge on the relationship between clinical GC dosing and cellular GC actions is addressed in Table 1. This table also contains data on so-called cytosolic GCR (cGCR)–mediated nongenomic actions discussed below, but there is currently only scattered information on dose-effect relationships. The specific nongenomic actions we describe below are not shown in Table 1, since their functional relevance is still not clear. More detailed information on the genomic and nongenomic actions of GCs are given below.

Table 1. Current knowledge on the relationship between clinical dosing and cellular actions of glucocorticoids

Terminology*	Clinical application†	Genomic actions (receptor saturation)‡§	Nongenomic actions§	
			Nonspecific	cGCR-mediated
Low dose (≤7.5 mg/day)	Maintenance therapy for many rheumatic diseases	+ (<50%)	–	?
Medium dose (>7.5 to ≤30 mg/day)	Initial treatment for primary chronic rheumatic diseases	++ (>50 to <100%)	(+)	(+)
High dose (>30 to ≤100 mg/day)	Initial treatment for subacute rheumatic diseases	++(+) (almost 100%)	+	+
Very high dose (>100 mg/day)	Initial treatment for acute and/or potentially life-threatening exacerbations of rheumatic diseases	+++ (almost 100%)	++	+(+?)
Pulse therapy (≥250 mg for 1 or a few days)	For particularly severe and/or potentially life-threatening forms of rheumatic diseases	+++ (100%)	+++	+(++?)

* Values represent mg of prednisone equivalent per day. See ref. 9 for further information.
† See ref. 9.
‡ See ref. 10.
§ cGCR = cytosolic glucocorticoid receptor; ? = unknown; – = not relevant; (+) = perhaps relevant, but of minor importance; + = relevant; +(+?) = relevant or perhaps even very relevant; +(++?) = relevant or perhaps even very or most relevant; ++ = very relevant; ++(+) = very relevant to most relevant; +++ = most relevant.

Genomic actions: classic and important, but do not explain everything

The important antiinflammatory and immunomodulatory effects of GCs are mediated predominantly by *genomic* mechanisms (Figures 1 and 2). Binding to cGCR ultimately induces ("transactivation") or inhibits ("transrepression") the synthesis of regulator proteins (11). The characteristics of the genomic mechanisms are as follows. First, they are physiologically relevant and therapeutically effective at all dosages, even very small ones (low-dose therapy). Second, the genomic action is slow; significant changes in regulator protein concentrations are not seen before 30 minutes because of the time required for cGCR activation/translocation, transcription, and translation effects. Third, the GC-induced synthesis of regulator proteins can be prevented by inhibitors of transcription (e.g., actinomycin D) or inhibitors of translation (e.g., cycloheximide). Fourth, between 10 and 100 genes per cell are directly regulated by GCs, but many genes are regulated indirectly through an interaction with transcription factors and coactivators (see below) (12). It is estimated that GCs influence the transcription of ~1% of the entire genome (13).

In the last few years, our in-depth knowledge of the genomic action of GCs has greatly increased. Their lipophilic structure and low molecular mass allow GCs to pass easily through the cell membrane and bind to the inactive cGCR (α-form [cGCRα]) (9).

Structure of the cGCR. The unactivated (unligated) cGCR is a 94-kd protein (Figure 3) retained in the cytoplasm as a multiprotein complex consisting of several heat-shock proteins (HSPs) including Hsp90, Hsp70, Hsp56, and Hsp40 (chaperones). Furthermore, the cGCR interacts with immunophilins, p23, and several kinases of the MAPK signaling system, including Src, which also acts as a molecular (co)chaperone (Figures 1 and 3) (11,14–16). The general function of molecular (co)chaperones is to bind and to stabilize proteins at intermediate stages of folding, assembly, translocation, and degradation. With regard to the GCRs, they also regulate cellular signaling, which includes stabilizing a specific conformational state of the GC that binds ligand with high affinity (17), the simultaneous opening of the steroid-binding cleft to access by steroid (18), and stabilizing the binding of GCRs to the promoter (19).

It has very recently become known that the first step in the assembly of the above-mentioned multiprotein complex is the ATP-dependent and Hsp40(YDJ-1)–dependent formation of a cGCR–Hsp70 complex that primes the receptor for subsequent ATP-dependent activation by Hsp90, Hop, and p23 (20). Figure 3 shows that the GCR consists of different domains that have distinct functions: an N-terminal, a DNA-binding domain, and a ligand-binding domain. The N-terminal harbors transactivation functions, especially within the so-called τ_1 region. Within the DNA-binding domain resides a motif common to DNA-interacting proteins, the zinc-finger motif, two of which are present. The ligand-binding domain consists of 12 α-helices, several of which take part in forming a hydrophobic ligand-binding pocket (16). The cGCR also contains another

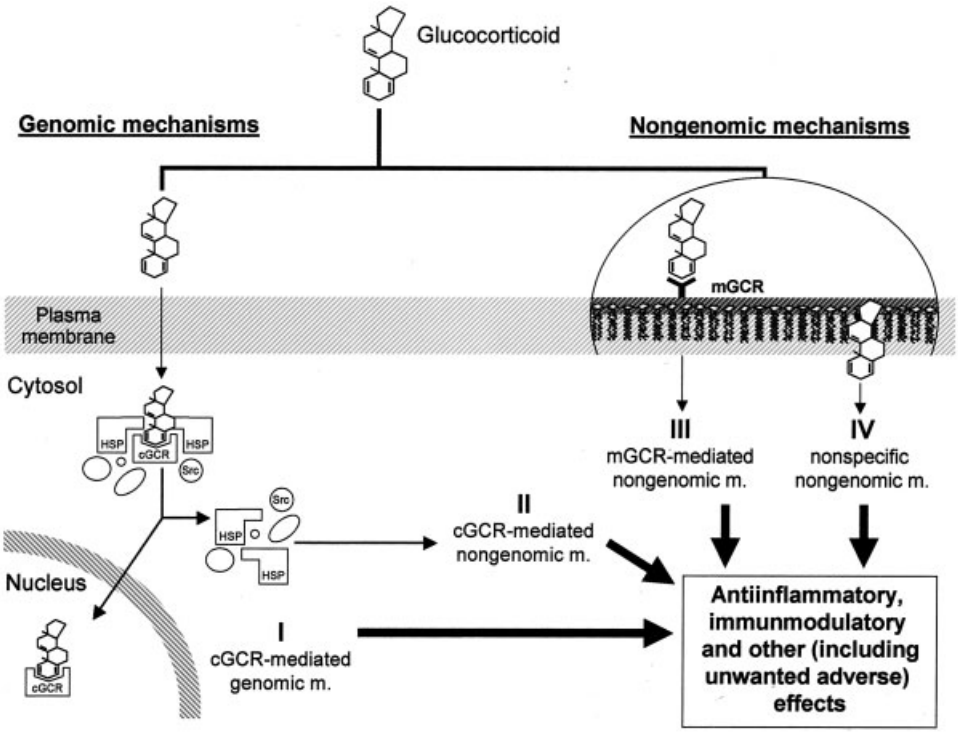

Figure 1. Mechanisms of the cellular actions of glucocorticoids. As lipophilic substances, glucocorticoids pass very easily through the cell membrane into the cell, where they bind to ubiquitously expressed cytosolic glucocorticoid receptors (cGCR). This is followed by either the classic cGCR-mediated genomic effects (**I**) or by cGCR-mediated nongenomic effects (**II**). Moreover, the glucocorticoid is very likely to interact with cell membranes either specifically, via membrane bound glucocorticoid receptors (mGCR) (**III**), or via nonspecific interactions with cell membranes (**IV**). HSP = heat-shock protein; m. = mechanism.

major transactivation region, τ_2, that can interact with the above-mentioned cofactors (Figure 3). Rapid shedding of Hsp90 molecules and other chaperones follows the binding of GC to its receptor. Translocation into the cell nucleus is thus possible, and there, the GC/cGCR complex finally binds as a homodimer to consensus palindromic DNA sites, the GC response elements (GREs) (11).

Translocation into the nucleus. It has been shown that the process of translocation occurs within 20 minutes and takes place much faster at 37°C than at lower temperatures (21). Furthermore, it has been suggested that hormone-directed recruitment of FK-506 binding protein 52 (FKBP52) and dynein to the GCR causes the transport of the GCR as a complex to the nuclear compartment (21). Depending on the target gene, transcription is thus either activated (transactivation via positive GRE) or inhibited (negative GRE) (Figure 2, mechanisms I and II). A well-known example of this process is the inhibition of cytokine synthesis.

Interactions with transcription factors. Besides the interactions of GC/cGCR complexes with GREs, the interaction of activated cGCR monomers with transcription factors is recognized as a further important genomic mechanism of GC action. Accordingly, although the GC/cGCR complex does not inhibit their synthesis, it modulates the activity of activator protein 1 (AP-1), NF-κB, and nuclear factor of activated T cells (NF-AT) (22–26). This leads to inhibition of the nuclear translocation and/or function of these transcription factors and, hence, to inhibition of the expression of many immunoregulatory and inflammatory cytokines. The following mechanisms are still under investigation (11): through interactions between the GC/cGCR complex and GRE in some types of cells, GCs induce the synthesis of IκB (a specific inhibitor of NF-κB) (Figure 2, mechanism I);

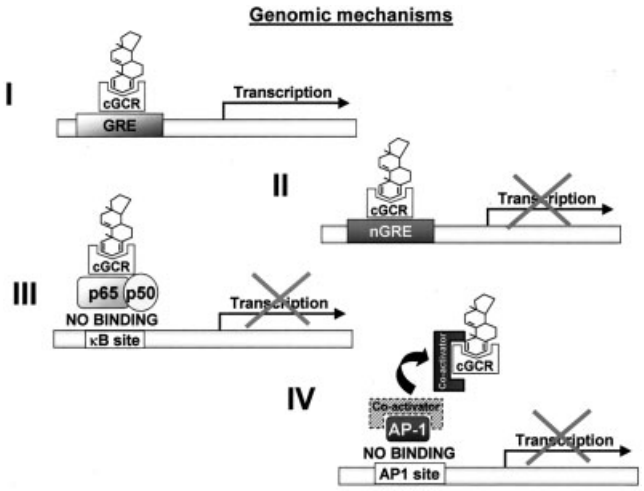

Figure 2. Genomic mechanisms of glucocorticoids. The different mechanisms by which the activated glucocorticoid receptor complex leads to the induction or inhibition of transcription and, finally, to the translation/synthesis of specific regulator proteins are illustrated. These features are detailed in the text. cGCR = cytosolic glucocorticoid receptor; GRE = glucocorticoid response element; nGRE = negative GRE; AP-1 = activator protein 1. Figure composed from information published in ref. 11.

the GC/cGCR complex undergoes protein–protein interactions with transcription factors through binding to their subunits (Figure 2, mechanism III), and this prevents their binding to DNA; and competition for nuclear coactivators arises between the GC/cGCR complex and transcription factors (Figure 2, mechanism IV).

Inhibition of transcription factor function and the resultant inhibition of protein expression are referred to as a transrepression mechanism. A large number of genes are regulated by this process. There are indications that many adverse clinical effects are caused by the transactivation mechanism (i.e., induced synthesis of regulator proteins), while many important antiinflammatory effects are mediated by the transrepression mechanism (i.e., inhibited synthesis of regulator proteins). This differential molecular regulation is the basis for current drug-discovery programs aimed at the development of dissociating cGCR ligands (see the section on selective glucocorticoid receptor agonists below) (2).

The cGCRβ isoform. With respect to the regulation of genomic glucocorticoid actions, it should be mentioned that an alternative splice variant of the cGCRα exists, the cGCRβ isoform. This isoform does not bind ligand and has been proposed to inhibit classic cGCRα-mediated transactivation of target genes. Recent research on the subject has provided structural insight, and a possible physical explanation for the lack of hormone binding and the dominant-negative actions of cGCRβ (27).

Posttranscriptional and posttranslational mechanisms. GCs also act through posttranscriptional and posttranslational mechanisms. The best-known examples are the reduction of cytokine messenger RNA half-life (e.g., via reduced levels of messenger RNA) and the mechanisms of GCR down-regulation (e.g., by reducing the stability of the GCR protein) (for more in-depth information, see ref. 28).

Nongenomic actions: now accepted after long debate

Some regulating effects of GCs arise within a few seconds or minutes (1,29–33). Such observations cannot be explained by the above-mentioned genomic actions because of the time required for their occurrence. *Nongenomic* mechanisms of action are responsible for these rapid effects. As a result of intensive research over

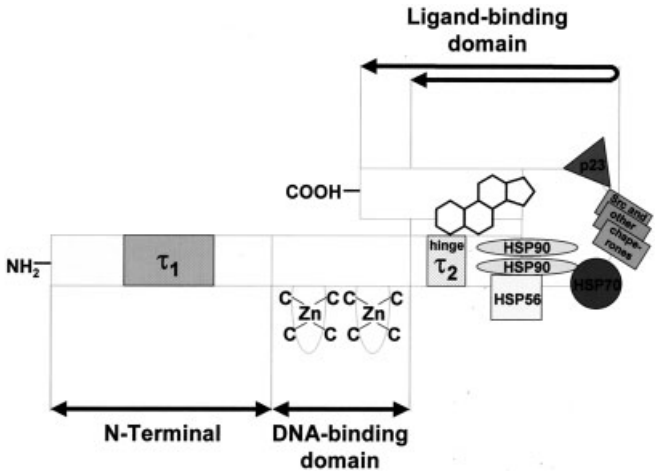

Figure 3. Structure of the cytosolic glucocorticoid receptor. The unactivated (unligated) cytosolic glucocorticoid receptor (cGCR) is a 94-kd protein that is retained in the cytoplasm as a multiprotein complex consisting of several heat-shock proteins (HSPs), including Hsp90, Hsp70, Hsp56, and Hsp40 (chaperones). Furthermore, the cGCR interacts with immunophilins, p23, and several kinases of the MAPK signaling system, including Src, which also act as molecular (co)chaperones. An important function of molecular (co)chaperones is to stabilize a specific conformational state of the glucocorticoid receptor, which binds ligand with high affinity (see text for details). The receptor protein itself consists of different domains: an N-terminal, a DNA-binding domain, and a ligand-binding domain. The N-terminal harbors transactivation functions, especially within the so-called τ_1 region. Another major transactivation region is τ_2, which can interact with the above-mentioned cofactors. Adapted, with permission, from ref. 13.

the last few years, the classic model of genomic actions can be considerably extended to include 3 different rapid *nongenomic* actions of GCs (1,29–33). These are discussed below.

The cGCR-mediated nongenomic actions: are chaperones more than chaperones? Once binding of GC molecules to the cGCR has taken place, not only do the classic genomic actions discussed above occur, but rapid nongenomic (or, non-nuclear) actions also occur. Croxtall et al (32) recently reported that epidermal growth factor–stimulated activation of cytosolic phospholipase A_2 with subsequent arachidonic acid release can be rapidly inhibited by dexamethasone. This effect is thought to be mediated by the occupation of cGCR, but not by changes in gene transcription. The reason for this assumption is that the observed effect is RU-486–sensitive (i.e., GCR-dependent), but actinomycin-insensitive (i.e., transcription-independent). Those investigators considered the above-mentioned chaperones or cochaperones of the multiprotein complex to act as signaling components and, therefore, as mediators of this effect.

Following glucocorticoid binding, the cGCR is released from this complex to mediate classic genomic actions. However, there is also a rapid release of Src and other (co)chaperones of the multiprotein complex that may be responsible for producing effects such as rapid inhibition of arachidonic acid release. In this context, Hafezi-Moghadam et al (33) recently reported cardiovascular protective effects of dexamethasone that could not be explained either genomically (because they occurred too quickly and could not be blocked by the transcription inhibitor actinomycin D) or nonspecific nongenomically (because they occurred at too low a dosage [100 n*M*]; see below). Those investigators suspected the mechanism to be binding of the GCs to the cGCR, leading to nontranscriptional activation of phosphatidylinositol 3-kinase, protein kinase Akt, and endothelial nitric oxide synthase (33).

Nonspecific nongenomic actions: do very high doses really help? In rheumatology, it is not uncommon to administer GCs at very high doses, e.g., by intraarticular injection or intravenous pulse therapy. Systemically administered doses of more than 100 mg of prednisone equivalent a day are regarded as "very high-dose" therapy. "Pulse therapy" is considered to be a specific therapeutic entity consisting of the administration of ≥250 mg of prednisone equivalent a day for 1 or a few days (9) (Table 1). Saturation of all cGCR is almost complete at a dosage of 100 mg of prednisone equivalent a day (10), such that the specificity (i.e., the exclusivity of receptor-mediated effects) is lost at high clinically relevant concentrations of GCs. *Nonspecific nongenomic* actions in the form of physicochemical interactions with biologic membranes occur, which probably contribute to the therapeutic success (1). A nonspecific intercalation of GC molecules into the cell membranes, altering cell functions by influencing cation transport through the plasma membrane and by increasing the proton leak of the mitochondria, is now being discussed by the scientific community as important mechanisms of GC actions. The resulting inhibition of calcium and sodium cycling across the plasma membrane of immune cells is thought to contribute to rapid immunosuppression and to a reduction in the activity of the inflammatory processes (1,30).

The use of such high doses in this way distinguishes rheumatologists (and also neurologists and traumatologists, for example) from other specialist physicians, and has led to critical discussions on the part of endocrinologists and pharmacologists. Are these high doses really necessary? Unfortunately, this is scientifically still an open question since the lack of randomized controlled trials has not allowed the development of evidence-based guidelines on this issue. However, in clinical practice, high-dose GC therapy is considered to be first-line treatment and has been used with clinical success in many situations.

The domains of high-dose GC therapy are acute exacerbations of life-threatening diseases and therapeutically resistant clinical conditions of various causes. One example is the treatment of systemic lupus erythematosus (SLE), where studies have shown that very high-dose GC therapy, such as pulse intravenous methylprednisolone therapy, is effective. However, these studies were mainly uncontrolled and retrospective. In their recent review of this issue, Badsha and Edwards (34) reported that intravenous pulses of methylprednisolone administered to patients with organ and/or life-threatening manifestations of SLE resulted in rapid immunosuppression. They concluded, however, that the gold standard of treatment, 1 gm/day for 3 consecutive days, "is associated with significant infectious complications, and lower doses may be just as useful" (34). Another example is the treatment of immune thrombocytopenia as a common manifestation of SLE, where high-dose GCs are typically (and mostly successfully) used, although comparative studies are lacking (35). Some further examples are given in Table 2.

In addition, there are many reports on the rapid, nongenomically mediated effects of mineralocorticoids, vitamin D, testosterone, progesterone, and estrogens.

Table 2. Examples of the successful use of high-dose glucocorticoids in various rheumatic and other diseases

Clinical observations/recommendations	Author, year (ref.)
Pulse methylprednisolone had strong inhibitory effects on proinflammatory mediators in peripheral blood, synovial fluid, and the synovial membrane in rheumatoid arthritis	Youssef et al, 1997 (36)
Pulse corticosteroid therapy significantly improved disease activity, physical functioning, and psychological well-being in patients with active rheumatoid arthritis	Jacobs et al, 2001 (37)
Glucocorticoid treatment effective for polyarteritis nodosa and microscopic polyangiitis with poor prognostic factors (15 mg/kg/day by intravenous pulse for 3 days, then 1 mg/kg/day orally for 3 weeks)	Guillevin et al, 2003 (38)
Intravenous pulse methylprednisolone (30 mg/kg/day) was highly effective in children with juvenile dermatomyositis	Fisler et al, 2002 (39)
Intravenous pulse methylprednisolone (1 gm/day for 3 days) is the recommended treatment for vasculitis in Behçet's disease	Kaklamani and Kaklamanis, 2001 (40)
High-dose intravenous pulse glucocorticoid is the treatment of choice for acute relapses in patients with multiple sclerosis	Grauer et al, 2001 (41)
Intravenous methylprednisolone at 30 mg/kg of body weight is established therapy for improving neurologic recovery after spinal cord injury	Kavanagh and Kam, 2001 (42)

We do not discuss here the nonspecific actions of any of these steroids because they are not used therapeutically in such high doses as are given in high-dose GC therapy. For GCs it is clear, however, that high concentrations are achieved in vivo. Table 3 shows this clearly with the example of methylprednisolone, a GC that is frequently used for high-dose intravenous therapy.

Several different approaches have been able to show that high, but clinically relevant, concentrations of methylprednisolone (as with the intravenous administration of the hemisuccinate) have immediate effects on immune cells. Intraarticular injections also bring high concentrations of GCs into contact with inflammatory cells at the site of inflammation, although precise statements about the local concentrations achieved are difficult to make because of the type of preparation (crystal suspension) that is most often used.

Specific nongenomic actions: and mGCR really do exist. Glucocorticoids may also cause *specific nongenomic* actions that are mediated through membrane-bound GCRs (mGCR). During the last few years, more evidence of the existence and function of membrane-bound receptors has become available for various steroids (including mineralocorticoids, gonadal hormones, vitamin D, and thyroid hormones) (29,31,32,48–51). With respect to glucocorticoids, mGCR were previously only known to exist in amphibian brains (52) and leukemia/lymphoma cells (53,54). However, a very recent article reported the physiologic existence of small numbers of mGCR on cell surfaces (55). With the use of highly sensitive immunofluorescence techniques, a significant expression of mGCR on human peripheral blood mononuclear cells (monocytes and B lymphocytes) obtained from healthy controls was demonstrated.

Table 3. Relationship between the dose and the plasma level of methylprednisolone*

Methylprednisolone administration	Plasma concentration		Author, year (ref.)
	In μg/ml	In moles/liter	
40 mg IV	~0.5	$\sim 0.1 \times 10^{-5}$	Szefler et al, 1986 (43)
80 mg IV	~1	$\sim 0.3 \times 10^{-5}$	Al-Habet and Rogers, 1989 (44)
10 mg/kg IV	~12	$\sim 3 \times 10^{-5}$	Derendorf et al, 1985 (45)
1 gm orally	Maximum 10	$\sim 3 \times 10^{-5}$	Hayball et al, 1992 (46)
1 gm IV	Maximum 22	$\sim 6 \times 10^{-5}$	Hayball et al, 1992 (46)
1.5 gm IV	Maximum 42	$\sim 10 \times 10^{-5}$	Defer et al, 1995 (47)
30 mg/kg of body weight IV, an established therapy for improving neurologic recovery after spinal cord injury (ref. 38)	Data not available, but considered to be >42	Data not available, but considered to be $>10 \times 10^{-5}$	Kavanagh and Kam, 2001 (42)

* This is a representative selection of studies that have determined the maximum plasma concentration of methylprednisolone achieved in humans in relation to the administered dose. In the conversions to moles/liter, a molecular weight of 374.48 gm/mole was taken for methylprednisolone. It is usually administered intravenously as the hemisuccinate ester (496.5 gm/mole). The maximum plasma concentrations of methylprednisolone hemisuccinate that have been measured are 2–10 times higher (44–46). IV = intravenously.

The monoclonal antibody used to detect this expression recognized not only cGCR, but also mGCR. This gave rise to the hypothesis that mGCR are probably variants of cGCR produced by differential splicing or promoter switching (55,56). It has also been found that immunostimulation with lipopolysaccharide increases the percentage of mGCR-positive monocytes; this can be prevented by inhibiting the secretory pathway with brefeldin A (55). It is concluded from these data that mGCR are actively up-regulated and transported through the cell following immunostimulation.

These in vitro findings are consistent with the clinical observation that in patients with rheumatoid arthritis, the frequency of mGCR-positive monocytes is increased and is correlated positively with disease activity (55). This observation may imply that mGCR play a role in the pathogenesis of disease; however, it is more likely that they are involved in negative feedback regulation. The demonstration of mGCR gives rise to certain questions that still have to be answered by further experiments. What are the functions of mGCR? Do they really mediate rapid nongenomic actions? Which actions are triggered by which signal cascades? How do the mGCR move into/onto the cell membrane?

New glucocorticoids in the pipeline

The various mechanisms of action provide interesting and sometimes very advanced starting points for the development of optimized GCs and GCR ligands. We will now take a brief look at these considerable developments.

Selective glucocorticoid receptor agonists. The existence of genomic component mechanisms of "transactivation" and "transrepression" provides the occasion for consistent developmental research on GCR ligands that predominantly cause transrepression, but not transactivation. As the basis for their research, Lin et al (57) took the previously discussed assumption that the anti-inflammatory properties of GCs are mostly due to repression of the AP-1– and NF-κB–stimulated synthesis of inflammatory mediators, whereas most of their adverse effects are associated with the transactivation of genes involved in metabolic processes. They therefore successfully sought to discover novel GCR ligands that have high repression but low transactivation activities. A compound, A276575, was found that (similar to dexamethasone) exhibits a high affinity for GCRs and potently represses interleukin-1α (IL-1α)–stimulated IL-6 production (57). However, in contrast to dexamethasone, A276575 induces little aromatase activity. Other novel, nonsteroidal GCR ligands are being developed which possess high repression activities against the production of inflammatory mediators, but have lower transactivation activities than do traditional steroids (for further information see for example, refs. 2 and 58).

Substances that cause a receptor conformation, preferring a GCR/protein interaction and not a GCR/DNA binding-dependent mechanism, are now being called "dissociating glucocorticoids" or selective glucocorticoid receptor agonists (SEGRAs) (2,58). At the moment, it cannot be reliably predicted whether SEGRAs will, as "improved glucocorticoids," enter the realm of clinical medicine in the near future. However, these novel developments are very interesting, and further in vivo investigations and preliminary clinical trials will have to be performed in order to define the safety/efficacy profile of SEGRAs (2).

The 21-aminosteroids (lazaroids). Methylprednisolone at high doses (30 mg/kg given intravenously) is an established therapy for improving neurologic recovery after spinal cord injury in humans (42). At these doses, nongenomic interactions with cell membranes (in this case, inhibition of lipid peroxidation) are therapeutically relevant. These neuroprotective effects are independent of its GCR actions (42). These findings stimulated the development of a group of glucocorticoid analogs, the 21-aminosteroids (lazaroids), that specifically inhibit lipid peroxidation without glucocorticoid or mineralocorticoid activity, thereby avoiding the complications of GC therapy (42,59). One representative of this class of drugs, tirilazad, has been shown to be neuroprotective (60) and to inhibit ultraviolet A–induced lipid peroxidation in human dermal fibroblasts (61). This drug is generally well tolerated; adverse reactions (such as local discomfort at the site of infusion and physical signs of local venous irritation), although commonly observed, are usually mild and transient. No research has yet been performed on their use in rheumatology, although it would be worthwhile investigating the use of 21-aminosteroids in conditions in which high-dose GCs are indicated.

Nitrosteroids, another novel class of glucocorticoids. Very recent experimental observations prompt the assessment of the clinical impact of another new class of glucocorticoid drugs, nitrosteroids, on rheumatoid arthritis and inflammatory bowel disease (62). Nitrosteroids are able to release low levels of nitric oxide (NO). They have been shown to be endowed with enhanced antiinflammatory properties (62,63) and reduced side effects (62,64). The prototype of these new steroids, 21-NO-prednisolone (or, NCX-1015), is much

more potent than prednisolone in models of acute and chronic inflammation, including type II collagen–induced arthritis (62,64). In contrast, an in vitro assay of bone resorption showed that NCX-1015 did not activate primary osteoclast activity, whereas prednisolone did. This lack of effect of NCX-1015 was chiefly due to NO. It has been suggested that posttranslational modification of GCR (tyrosine nitration) by this novel nitrosteroid is one reason for its enhanced antiinflammatory activity (65). Another important finding is that NCX-1015 potently stimulates IL-10 production, suggesting that nitrosteroids induce a regulatory subset of T cells that negatively modulate inflammation (66). However, more studies are needed to confirm that nitrosteroids will be effective as antiinflammatory agents in clinical practice.

Long-circulating liposomal glucocorticoids. As we stated above, the antiinflammatory effectiveness of GCs can be improved by the additional benefits of the nongenomic actions of high concentrations. On this basis, the successful use of long-circulating liposomal GCs has recently been reported (67,68). In rats with experimental autoimmune encephalitis, it was shown that GC-containing liposomes accumulate at sites of inflammation, reaching ultra-high concentrations ($>10^{-5}$ moles/liter for at least 18 hours), and are therefore therapeutically superior to conventional high-dose intravenous GC therapy (67).

In another recently published study (68), the same group of investigators reported the successful use of this new therapeutic option in rats with adjuvant-induced arthritis. The investigators observed that a single injection of 10 mg/kg of liposomal prednisolone phosphate resulted in complete remission of the inflammatory response for almost a week. In contrast, the same dose of unencapsulated prednisolone phosphate did not reduce inflammation, and only a slight effect was observed after repeated daily injections. It was concluded in both studies that preferential GC delivery to the site of inflammation leads to very high GC concentrations in, for example, the inflamed joint (accompanied by low plasma concentrations, with perhaps a lower rate of side effects), which is the key factor that explains the strong therapeutic effect that was observed (67,68). These are very promising developments that aim at using the broad spectrum of the therapeutically relevant genomic and nongenomic actions of GCs preferentially at sites of inflammation.

Conclusion

In summary, the results of research over the last few years have greatly increased our knowledge of glucocorticoids as the best antiinflammatory agents available to date. In particular, new findings on the actions of the occupation of cytosolic GC receptors on intracellular signaling, transcription processes, and gene expression, as well as the existence of membrane-bound glucocorticoid receptors and the information on dose-effect relationships, have stimulated intensive research activity with the aim of bringing this increased knowledge from scientific research into clinical use as quickly as possible. The new GCR ligands and the administration of liposomes are very promising approaches that, hopefully, will soon be available in clinical practice to improve the risk/benefit ratio and well-being of patients being treated.

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