New Glucocorticoids on the Horizon: Repress, Don't Activate!

IN-HO SONG, RALF GOLD, RAINER H. STRAUB, GERD-RÜDIGER BURMESTER, and FRANK BUTTGEREIT

ABSTRACT. Glucocorticoids are highly effective drugs; their immunosuppressive and antiinflammatory actions are used in the treatment of many rheumatic and other inflammatory diseases. However, their use is sometimes considerably limited by numerous adverse reactions. For this reason, great efforts have been made in recent years to develop innovative glucocorticoids or glucocorticoid receptor ligands that have improved therapeutic effect/adverse reaction ratio. We summarize the position and critically discuss the following products that are currently under development: (1) selective glucocorticoid receptor agonists (SEGRA or dissociating glucocorticoids); (2) nitrosteroids; and (3) long-circulating liposomal glucocorticoids. Finally, we describe the state of research on membrane-bound glucocorticoid receptors as possible further targets for specific glucocorticoid actions. (J Rheumatol 2005;32:1199–207)

Key Indexing Terms:
GLUCOCORTICOIDS GENOMIC AND NONGENOMIC MECHANISMS
CYTOSOLIC AND MEMBRANE-BOUND GLUCOCORTICOID RECEPTOR
TRANSACTIVATION TRANSREPRESSION NITRIC OXIDE

Because of their strong immunosuppressive and antiinflammatory actions, glucocorticoids are indispensable for the treatment of acute and chronic inflammatory diseases, especially inflammatory rheumatic conditions such as rheumatoid arthritis or collagen vascular diseases. Nevertheless, particularly in high doses over long periods, the use of glucocorticoids is limited by numerous, sometimes irreversible, adverse reactions. These include Cushing's syndrome, osteoporosis, myopathy, increased susceptibility to infection, and adverse effects on the eyes and skin[1-8].

The high therapeutic potency of glucocorticoids on the one hand and their potential to cause adverse reactions on the other have stimulated great efforts in recent years to develop new glucocorticoids and glucocorticoid receptor ligands with an improved therapeutic effect/adverse reaction ratio. We describe the state of development with respect to selective glucocorticoid receptor agonists (SEGRA)[9], the substances known as nitrosteroids[10], and long-circulating liposomal glucocorticoids[11]. Finally, we review current research on membrane-bound glucocorticoid receptors as possible further targets for specific glucocorticoid actions.

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Accepted for publication January 13, 2005.

New drug developments based on genomic glucocorticoid actions

The genomic mechanism of action of glucocorticoids. As lipophilic molecules, glucocorticoids are able to diffuse through cell membranes into the cytosol and bind to cytosolic glucocorticoid receptors (cGCR; Figure 1). Two activated glucocorticoid receptor complexes combine (dimerization) and are translocated into the cell nucleus, where they bind to promoter regions of certain genes (referred to as glucocorticoid response elements), thus inducing the synthesis of not only antiinflammatory proteins (e.g., lipocortin 1, IκB) but also metabolically important regulator proteins (e.g., gluconeogenesis enzymes). This process is known as "transactivation" (Figure 1). In addition, glucocorticoids induce genomic actions referred to as "transrepression" (Figure 1). This term is taken to mean that monomers of glucocorticoid molecules and cGCR interact with transcription factors such as glucocorticoid receptors apolipoprotein-1 (AP-1) and nuclear factor-κB (NF-κB) by direct protein–protein interaction and thus have an inhibitory effect on their function. In this way, for example, the synthesis of proinflammatory cytokines [e.g., tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), IL-2] and prostaglandins [inhibition of the expression of cycloogenase-2 (COX-2), phospholipase A_2] is reduced.

In recent years it has become clear that a large number of the desirable antiinflammatory and immunomodulating actions of glucocorticoids are due to transrepression, while a large proportion of the adverse reactions are due to transactivation. For this reason, a systematic search has been

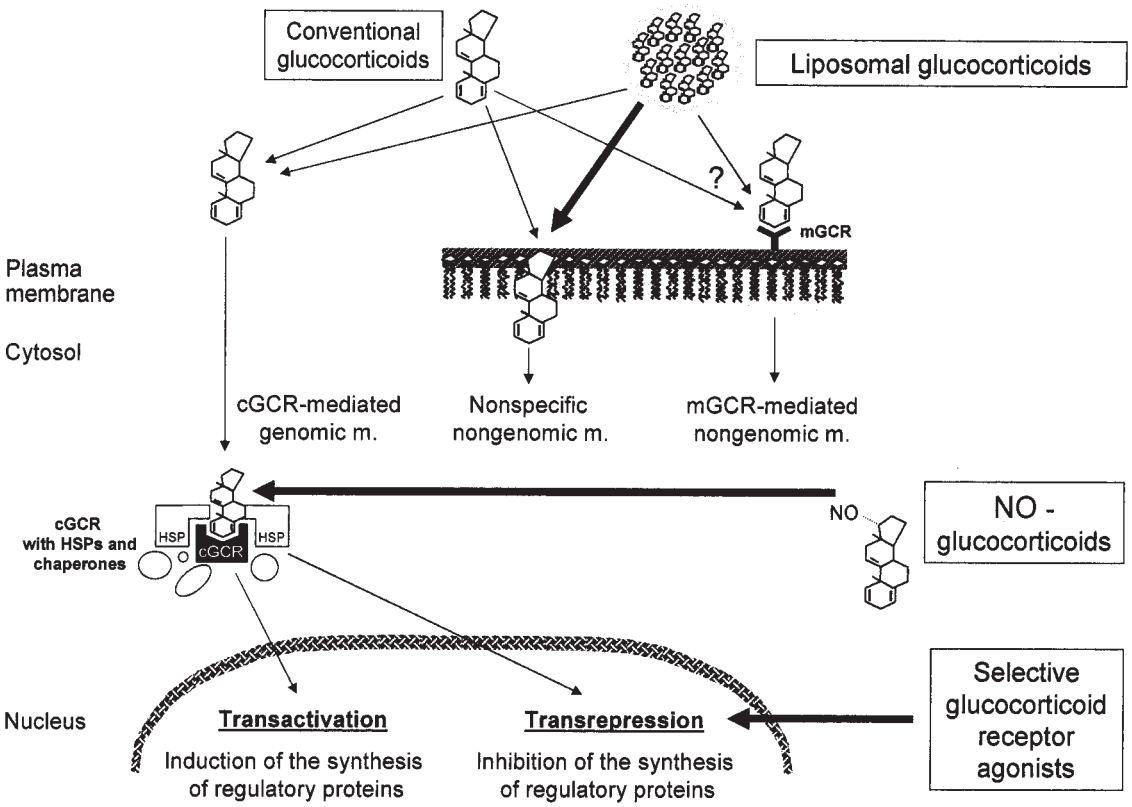

Figure 1. Mechanisms of action of conventional and new glucocorticoids: Diffusion of conventional glucocorticoids, NO-glucocorticoids and selective glucocorticoid receptor agonists (SEGRA) through the cell membrane, binding to the cGCR, translocation into the cell nucleus. In the nucleus there is either (1) transactivation: binding to DNA and induction of antiinflammatory proteins, e.g., lipocortin-1, IκB, and mediators of adverse effects; or (2) transrepression: inhibition of inflammatory proteins such as AP-1 and NF-κB by direct protein–protein interaction and reduced expression of proinflammatory cytokines (e.g., IL-1, IL-6, TNF-α) and prostaglandins (phospholipase A_2, COX-2). SEGRA have antiinflammatory effects through transrepression, but they cause fewer adverse reactions via transactivation. NO-glucocorticoids also bind to the cGCR, but they also slowly release NO, with improved therapeutic effects. Glucocorticoid-containing liposomes (liposomal glucocorticoids) accumulate selectively at the site of inflammation; consequently, very high concentrations ($> 10^{-5}$ M) of conventional glucocorticoid drugs such as prednisolone are achieved. cGCR: cytosolic glucocorticoid receptor; mGCR: membrane-bound glucocorticoid receptor, HSP: heat shock proteins, m: molecule.

made for substances known as "dissociating glucocorticoids." These innovative steroidal or nonsteroidal molecules bind selectively to the glucocorticoid receptor and induce the transrepression process as described. However, they have only a very limited ability to induce transactivation[12-20]. Another term for these substances therefore is "selective glucocorticoid agonists" (SEGRA). Reports published at the end of the 1990s based on the results of studies on transgenic mice[21,22] provided the incentive to search for SEGRA.

Selective glucocorticoid receptor agonists. At first there were reports of substances (e.g., RU24858, RU24782, and RU40066) that, although showing good dissociation *in vitro,* were not convincing *in vivo*[23-25]. For example, Belvisi, *et al* used a rat model (Sephadex-induced lung edema) to show that RU24858 exhibits an antiinflammatory activity similar to budesonide and prednisolone (the transrepression effect). However, there was no differentiation on quantitative osteopenia in the rat femur model of glucocorticoid osteopenia (the transactivation effect)[24]. To determine the effect on osteoblast formation the investigators measured osteocalcin, alkaline phosphatase, acid phosphatase, and bone-specific tartrate-resistant acid phosphatase[24]. It turned out that either treatment with the test compound RU24858 or with the conventional glucocorticoids resulted in a dose-related inhibition of serum osteocalcin concentrations and in a dose-dependent decrease in the above noted bone enzyme markers. Histopathologically, administration of each of the test substances over a 7-day period was associated with hypoplasia and loss of cellularity within the proliferative zone of the femur growth plate. Moreover, there was also no differentiation in the ability to induce systemic changes (e.g., body weight, thymus involution)[24]. These results suggested that *in vitro* separation of transrepression from transactivation activity does not always translate into an improved benefit/side effect ratio *in vivo*[24,25].

However, since then an increasing number of selective

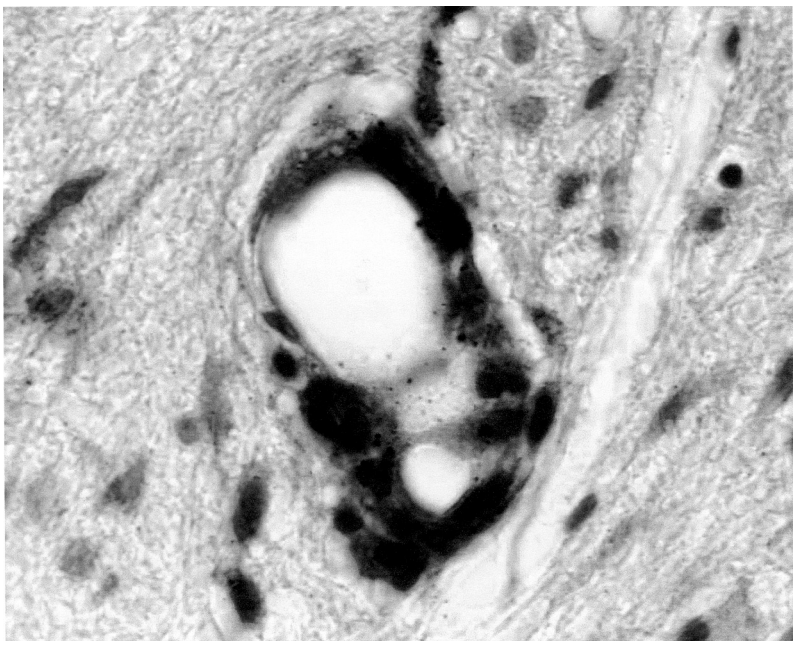

Figure 2. Perivascular microglial cells/macrophages in which silver-labeled liposomes can be seen, taken up by the macrophages (Lewis rat autoimmune encephalomyelitis model, spinal cord, original magnification ×1000). Kindly provided by J. Schmidt, Department of Neurology, University of Würzburg, Würzburg, Germany, and R. Gold.

dissociating glucocorticoids have been described. Among these are some very promising substances including A276575[26], ZK 216348[9], and N-arylpyrazolo [3,2-c]-based cGCR ligands[27]. In contrast to these selective dissociating glucocorticoids there are selective nondissociating substances, including AL-438; more detail on these molecules is given below.

The selective dissociating glucocorticoid receptor agonist A276575. Lin, *et al* developed a new series of GR ligands[26], including A276575 and its 4 enantiomers. *In vitro* experiments (H_3 radioligand binding assays) have shown that A276575 binds the cGCR equally as strongly as dexamethasone. The antiinflammatory properties of A276575 and its 4 enantiomers have been convincingly proven in cell cultures on the basis of (1) inhibition of IL-1ß-stimulated IL-6 production, and (2) reduction of concanavalin-A-induced proliferation of peripheral blood mononuclear cells. However, the fact that A276575 and its enantiomers clearly have less transactivation effect than dexamethasone was decisive. In corresponding studies with reporter gene constructs (MMTV-GRE assays), it was established that the new substances showed less than 5% of the ability of dexamethasone with respect to transactivation. A276575 was even able to abolish the transactivation effects of dexamethasone. It is interesting that the 2 (–) enantiomers of A276575 have an affinity for cGCR up to 30 times greater than the (+) enantiomers.

The selective dissociating glucocorticoid receptor agonist ZK 216348. Interesting animal model data have been published recently. Schäcke, *et al* were able to identify the substance ZK 216348 as a new nonsteroidal SEGRA[9]. Binding assays have shown that this substance has a high cGCR-binding affinity similar to that of dexamethasone and 3 times the affinity of prednisolone. This induces an effective antiinflammatory action, which the authors first demonstrated *in vitro* on the basis of the inhibition of TNF-α and IL-12 synthesis. The ability of ZK 216348 for transactivation was quantified by measuring tyrosine aminotransferase induction. This showed that the new substance is about 60 times weaker than prednisolone and over 300 times weaker than dexamethasone with respect to transactivation. These cell culture data were impressively confirmed by animal experiments. The antiinflammatory actions were observed *in vivo* in the croton oil-induced ear inflammation model in mice and rats. Further *in vivo* studies showed that ZK 216348 indeed has a very much better adverse reaction profile than prednisolone. This evidence was based on the induction of skin atrophy, increased body weight, apoptosis of immune cells (measured by weight of thymus and spleen), and increased blood glucose. ZK 216348 and prednisolone had similar effects on the hypothalamus-pituitary-adrenal cortical axis (ACTH measurements).

Selective dissociating N-arylpyrazolo [3,2-c]-based cGCR ligands. Ali, *et al* recently synthesized and described a series of potent and selective nonsteroidal ligands for the human cGCR[27]. These ligands are N-arylpyrazolo [3,2-c]-based. It was shown *in vitro* that a number of structural motifs can replace the conventional C- and D-rings of steroidal gluco-

corticoids, while still maintaining significant activity at the cGCR. These analogs exhibited strong affinity for the human cGCR and an interesting partial agonist profile in functional assays of transactivation (induction of tyrosine aminotransferase and glutamine synthetase) and transrepression (inhibition of IL-6). However, further experiments must be completed before a claim of dissociation can be made for these novel cGCR ligands.

The selective, nondissociating cGCR agonist AL-438. Miner and Coghlan, *et al* recently described a new substance, AL-438, which exhibits an altered gene regulation profile, able to repress and activate only a subset of genes normally regulated by glucocorticoids[28,29]. The investigators performed transfection studies showing that AL-438 can repress TNF/IL-1ß-induced E-selectin promoter activity as well as IL-1ß-induced IL-6 expression. However, the drug was unable to inhibit osteocalcin as efficiently as prednisolone. These data were consistent with the effects noted in osteoblast cell cultures. Further, *in vivo* studies revealed that AL-438 has similar antiinflammatory activity compared to prednisolone in a model of acute inflammation (carrageenan-induced paw edema assay in the rat) and chronic inflammation (adjuvant-induced arthritis in the rat), while not causing hyperglycemia or inhibiting bone mineral apposition in an animal model. It is suggested as the underlying mechanism of action that AL-438 is unable to efficiently interact with the coactivator PGC-1 (peroxismal proliferator-activated receptor-activated receptor gamma coactivator-1), which plays a critical role in glucocorticoid-mediated stimulation of gluconeogenesis. In summary, these data are challenging the concept that selective glucocorticoid action can only be achieved by dissociating transactivation and transrepression.

Selective glucocorticoid receptor modulators. Recently, Link, *et al* described a new class of nonsteroidal selective glucocorticoid receptor modulators (SGRM) typified by N-{3-[benzyl-(4-chloro-2-fluoro-benzyl)-amino]-2-methyl-phenyl}-methanesulfonamide 19[30]. After successful testing in binding assays against a panel of human nuclear hormone receptors, functional activity was proven in a reporter cell line genetically engineered to express human cGCR, the glucocorticoid response element, and a reporter gene encoding a secreted form of alkaline phosphatase. Nonsteroidal selective glucocorticoid receptor modulators like N-{3-[benzyl-(4-chloro-2-fluoro-benzyl)-amino]-2-methyl-phenyl}-methanesulfonamide 19 undergo rapid metabolic decomposition leading to poor pharmacokinetics. In order for them to have *in vivo* utility, potentially through inhibition of the expression of key enzymes that regulate hepatic glucose production, the metabolic stability of this series will first have to be improved[30].

NO-glucocorticoids (nitrosteroids). NO-glucocorticoids represent another very interesting and promising effort to develop optimized glucocorticoids (Figure 1). Behind this development is the fact that, in addition to the numerous other actions of nitric oxide (some proinflammatory), it also has antiinflammatory effects, especially with slow release[10]. These antiinflammatory effects of NO include, for example, reduced leukocyte adhesion to the endothelium, reduced leukocyte extravasation, diminished mast cell activation processes, and suppression of the synthesis of inflammatory mediators[31-36]. Coupling NO to other antiinflammatory substances allows a synergistic effect, which has already been established for other NO-releasing drugs (e.g., NO-Aspirin)[37,38].

NO-glucocorticoids (nitrosteroids) are glucocorticoid derivatives coupled with an aliphatic or aromatic linker molecule that binds NO with an ester bond. NCX-1015 (prednisolone 21- [(4'-nitro-oxymethyl)benzoate] and NCX-1004 (hydrocortisone 21-[(4'-nitro-oxy)butyrate]) are examples of NO-glucocorticoids. NO can be released from NCX-1015 by cleavage of the ester bond within 60 minutes and then starts to develop its antiinflammatory actions; this release is relatively slow. In contrast, nitroprusside, for example, releases NO in as little as 5 minutes' time.

Peretti and coworkers have recently done more detailed research into the antiinflammatory actions of NO-glucocorticoids[10,38,39]. They have demonstrated the accumulation of NO by ester cleavage after injection of NCX-1015 in the mouse peritoneum. Measurements using radioactively labelled [^{3}H]dexamethasone further revealed the binding of nitrosteroids such as NCX-1015 to cGCR, and finally showed that nitrosteroids have a much stronger antiinflammatory effect than prednisolone in a mouse peritonitis model (based on measurements of the inhibition of neutrophil extravasation)[38]. In further experiments, Paul-Clark, *et al* again confirmed the more potent antiinflammatory effects of NCX-1015 in comparison with prednisolone. In air-pouch granulomas in the mouse, the new substance inhibited mononuclear and polymorphonuclear cell migration more strongly[38]. In collagen type II-induced arthritis in rats, there was a greater reduction of joint swelling and IL-1 production[39]. Finally, animal models have provided evidence that NCX-1015 induces less osteoporosis than prednisolone. This conclusion was reached from findings of reduced osteoclast activation and lower serum pyridinoline concentrations[39].

In animal models of experimental colitis, NCX-1015 has already been shown to have good antiinflammatory effects[40]. In further studies, nitrosteroids have been found to be more potent bronchodilators than conventional glucocorticoids[41].

The observed improvement of the therapeutic effect profile of glucocorticoids due to NO is in agreement with observations made with other NO-donating drugs (NODD). Besides nitrosteroids, a large number of compounds that can release NO are being researched for various indications[42]. One example is NO-Aspirin, which has been reported to

have favorable cardiovascular effects on atherosclerosis and hypercholesterolemia, and in myocardial infarctions and arrhythmias[43-45], but with a low risk of ulceration[46]. Further examples are (1) NO-paracetamol (enhanced analgesic effect)[47]; (2) NO-flurbiprofen in the treatment of osteoporosis, incontinence, and neurodegenerative disorders[42,48]; (3) NO-mesalamine for chronic inflammatory bowel disease (enhanced antiinflammatory effects)[49]; (4) NO-ursodesoxycholic acid in portal hypertension[50]; and (5) NO-nonsteroidal antiinflammatory drugs in the prevention of colonic carcinoma[51].

In NO-glucocorticoids, NO has a synergistic antiinflammatory effect with the glucocorticoids. The following mechanisms are being discussed in this context, (1) a post-translational tyrosine nitration of cGCR and (2) the stimulation of IL-10 production, that suggest induction of regulatory T cells and thus modulation of inflammation[39]. The results to date of the improved therapeutic effect/adverse reaction ratio of these substances in comparison with conventional glucocorticoids are very promising. However, further investigations are necessary to confirm the benefits of these substances as new drugs in clinical practice, e.g., in the treatment of patients with arthritis.

New drug developments based on nongenomic glucocorticoid actions

Apart from the genomic actions described above, glucocorticoids also have nongenomic actions of rapid onset (Figure 1). The following mechanisms are currently under discussion: (1) mediated by cGCR (for clarity, not depicted in Figure 1); (2) nonspecific interactions with biological membranes (nonspecific nongenomic effects); and (3) mediated by membrane-bound glucocorticoid receptors (mGCR; specific nongenomic effects)[15,52-57]. The nonspecific nongenomic effects that are seen in particular with high concentrations provide the theoretical basis for interesting new developments that will be discussed in detail in the following sections.

Long-circulating liposomal glucocorticoids. This new development is based on the fact that glucocorticoids in high, yet clinically relevant concentrations induce the above-mentioned nonspecific nongenomic effects (Figure 1). Effects of this kind can be seen clearly with glucocorticoid concentrations of 10^{-6} to 10^{-5} M. High dose intravenous therapy or intraarticular injections achieve such concentrations when there are significant effects of physicochemical interactions of the glucocorticoid molecules with biological membranes[15,52-54]. Intercalation of the glucocorticoid molecule into the biological membranes is probably responsible for the effects on ATP-producing (mitochondrial respiratory chain) and ATP-consuming processes (e.g., cation transport, phospholipid turnover). These actions influence the function of immune cells by suppression: the activity of previously activated immune cells is inhibited, while the potential activity of quiescent cells is reduced[15]. These high dose effects are therefore clinically relevant. They appear in addition to the genomic actions that, at doses above 100 mg prednisone equivalent/day, are already at a maximum because of almost complete receptor occupancy. These findings have already been taken into account with the introduction of lazaroids (21-aminosteroids such as tirilazad). These substances intercalate into biological membranes but do not bind to the glucocorticoid receptors. They therefore induce nonspecific nongenomic, but not genomic, glucocorticoid actions[58]. Reduction in membrane fluidity leads to stabilization of membranes, to inhibition of destructive lipid peroxidation, and thus to the prevention of oxidative cell damage without the classical adverse effects of glucocorticoids or mineralocorticoids[59,60]. These results have been confirmed in many *in vitro* and *in vivo* studies[61-63]. Tirilazad is used primarily in neurotraumatology.

The actions of 21-aminosteroids on rheumatic diseases have not yet been investigated systematically. The reason is probably that the genomic effects are very much needed in the treatment of rheumatic conditions. Glucocorticoids naturally offer genomic actions. The problem with glucocorticoids, however, is that the high doses necessary to induce the therapeutically desirable nongenomic effects can be administered for only short periods ("pulse therapy"). A further problem with the systemic administration of glucocorticoids is that they are very quickly removed from the circulation. Where is the way out of this dilemma?

A very interesting attempt to solve the problem is drug targeting, with liposomes as the carrier system for glucocorticoids. Liposomes, first discovered more than 40 years ago[64], are small vesicles, roughly 100 nm in size, consisting of a lipid bilayer and a hydrophilic core. Substances such as glucocorticoids can be enclosed in these vesicles (drug carrier; Figure 1). Binding of hydrophilic polymers such as polyethylene glycol (PEG) to the surfaces of liposomes prevents their rapid breakdown by the mononuclear phagocyte system in the liver and spleen[65-67]. The half-life of PEG liposomes in the circulation is about 50 hours ("long-circulating") and they are effective over a correspondingly long period[68,69].

There are 3 aspects of particular importance for the newly developed glucocorticoid-containing liposomes: (1) they accumulate selectively at the site of inflammation, where there is an increased permeability of the local vascular endothelium and the presence of activated macrophages[70,71]. (2) This means that very high concentrations (> 10^{-5} M) are achieved at the site of inflammation for several hours, which leads to 100% utilization of the genomic actions and, in addition, significant therapeutically beneficial nongenomic actions. (3) Although the systemic glucocorticoid concentrations (plasma levels) remain relatively high, they give rise to fewer adverse reactions because the steroid is encapsulated in the liposome.

Investigations to date have used prednisolone encapsulated in the liposomes, as methylprednisolone liposomes have proved not to be stable[72,73]. Schmidt, *et al* recently used prednisolone liposomes to show the success of this innovative application in experimental autoimmune encephalomyelitis (EAE) in Lewis rats[11] (Figure 2). Prednisolone liposomes were compared to free prednisolone (and free methylprednisolone) according to an established protocol used in previous studies[72-74]. The authors showed that the prednisolone liposomes accumulate at the site of encephalitis, reaching very high local concentrations that reduce, for example, the infiltration of immune cells into the area. Not only was apoptosis of T cells found to be increased, but also the number of macrophages was reduced *in situ*. The latter effect is seen to a far greater extent than that achieved with 5 times the dose of free methylprednisolone. It should be stressed, however, that results from the rat EAE model should not be over-interpreted in terms of clinical efficacy and safety in humans.

The first publications on the use of long-circulating liposomal glucocorticoids in arthritis have recently appeared[75,76]. In animal models of mouse collagen type II-induced arthritis, Metselaar, *et al*[76] showed that a single injection of 10 mg/kg liposomal prednisolone phosphate results in a strong and sustained (1 week) resolution of joint inflammation. The same dose of unencapsulated prednisolone phosphate was only slightly effective after repeated daily injections. Using gold labelled liposomes it has also been shown that within the inflamed joint (1) glucocorticoid-containing liposomes accumulated in the synovial lining and in the surrounding blood vessels, whereas in an unaffected joint only a few liposomes were detectable; and (2) that there was only a negligible loss of cartilage in inflamed joints of the liposome treated group, whereas there was highly damaged cartilage in the control group[76]. To summarize, long-circulating liposomal glucocorticoids represent intelligent targeting of the target structures. It seems that it is indeed possible to improve the therapeutic effect/adverse reaction ratio greatly by consistent application of this research. The first animal experimental results are very encouraging. In further investigations both the lipid shells and the enclosed steroids will be optimized. Hopefully the data collected from use in human subjects will show similarly favorable therapeutic effects to those from animal models so that these drugs can rapidly be introduced into clinical practice.

Finally, we will mention that high glucocorticoid concentrations can also be achieved locally by using so-called "soft steroids." Soft steroids are active entities or prodrugs designed for delivery near their site of action, to exert their effect and then to undergo controlled and predictable metabolism to inactive metabolites, thus reducing systemic exposure and the likelihood of unwanted side effects[77,78]. For example, loteprednol etabonate, an inactive-metabolite soft steroid, has been accepted for the treatment of ophthalmic disorders and is now in phase III trials of clinical development for its effects on airway inflammation[79]. Ciclesonide, a prodrug soft steroid, is a nonhalogenated inhaled steroid ester prodrug that is metabolized intracellularly to form the active entity, which then binds to the cGCR. Ciclesonide has shown efficacy virtually without side effects in a once-daily formulation in patients with asthma and is being developed for the treatment of both asthma and chronic obstructive pulmonary disease.

mGCR as targets for new glucocorticoids?

As stated, glucocorticoids may also cause specific nongenomic actions mediated through membrane-bound glucocorticoid receptors (mGCR; Figure 1). These receptors have been described in amphibian brains[80] and leukemic/lymphoma cells[81-83]. We suspected that mGCR, as with other important molecules, are also physiologically expressed on cell surfaces, but in such small numbers that they are not detected by conventional techniques. Indeed, we have recently identified mGCR by the use of highly sensitive immunofluorescence[84]. This new method uses antibody-conjugated magnetofluorescent liposomes. The intensity of the fluorescent signal is thus increased a thousand-fold compared with conventional methods and allows 50–100 target antigens per cell to be identified with certainty. Using this technique we were able to observe by immunofluorescence and by a fluorescence imaging technique for the first time a significant expression of mGCR on human peripheral blood mononuclear cells from healthy controls, which was undetectable by conventional technologies. Up to 9.2% of monocytes and up to 12.3% of B lymphocytes, but not T lymphocytes, were found to be positive for mGCR[84]. The monoclonal antibody used for the detection recognized not only cGCR but also mGCR. This gave rise to the hypothesis that both these receptor proteins are derived from the same transcript. On this assumption, there would be only one mRNA that, taking splice variations and post-translational editing into account, produces proteins that end up as either cGCR in the cytosol or as mGCR on/in the membrane. Our experiments on overexpression of a his-tagged cGCR showed no increased mGCR expression on the surface. We therefore conclude that mGCR are not simply cGCR that have been transported to the surface unchanged. It is suggested that mGCR are probably variants of cGCR produced by differential splicing or promoter switching. Alternatively, the mGCR is produced from the cGCR by post-translational editing alone; however, this does not occur spontaneously, but is regulated.

We have also found that immunostimulation with lipopolysaccharide increases the percentage of mGCR-positive monocytes (but not the number of mGCR per cell); this can be prevented by inhibiting the secretory pathway with brefeldin A[84]. We concluded from this that mGCR are

actively upregulated and transported through the cell following immunostimulation. These observations gave rise to the suspicion that more monocytes may be mGCR-positive when there is disease-related increased activity of the immune system. We tested this hypothesis in a clinically characterized cohort of patients with rheumatoid arthritis. Our data indeed showed a strong positive correlation between the frequency of mGCR-positive monocytes and various disease activity indicators[84]. This observation may imply that mGCR play a role in the etiopathogenesis of disease. However, it is more likely they cause negative feedback regulation in the following sense: immunostimulation (or high disease activity) results in an induced mGCR expression on immune cells such as monocytes. This in turn leads to a significantly higher percentage of cells undergoing mGCR-mediated glucocorticoid-induced apoptosis[83], which ameloriates the activity of the immune system. Should this prove to be correct, this could provide a new approach to glucocorticoid development (Figure 1). Drugs binding selectively to the mGCR may in future be of therapeutic value, but the functions of mGCR must first be investigated in detail.

In the field of "good old glucocorticoids" developments are taking place that are potentially very exciting. Even if only one of the innovations described here comes to fruition, greatly improved glucocorticoids will be available to us. If we could use glucocorticoids in high doses for an unlimited length of time, much of the concern about the treatment of our patients with rheumatic disease would be eliminated. This is not possible due to the potential for adverse reactions, so each development that may improve the benefit/risk ratio is worthwhile. Current efforts with SEGRA, nitrosteroids, and long-circulating liposomal glucocorticoids are steps in the right direction. We hope that positive study results in humans will allow these promising developments to be rapidly transformed into clinical benefit.

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