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GR Ligands: Can We Improve the Established Drugs?

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Introduction

'Relief without side effect' was the heading for a recent news item[1] in reference to a study conducted by Haegeman and co-workers from the University of Ghent. In their report, they describe the binding of a nonsteroidal compound to the glucocorticoid receptor (GR).[2] Compound A (Figure 1), as it has been named, was

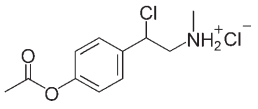

Figure 1. Structure of compound A.

claimed to have anti-inflammatory activity without some of the side effects normally associated with glucocorticoids (GCs). Compound A is a stable analogue of the hydroxyphenyl aziridine precursor found in the Namibian shrub *Salsola tuberculatiformis* Botschantzev, and exhibits a so-called dissociated or selective profile. What does the concept of a dissociated or selective GR ligand or modulator mean?[3]

GCs have been on the market for more than 50 years and represent the most effective therapy for acute and chronic inflammatory disorders including allergic diseases. However, their outstanding therapeutic effects are often accompanied by severe and sometimes irreversible side effects, such as diabetes mellitus, particularly when they are applied systemically. Thus, there is a real need for compounds with a decreased side-effect profile but which retaining the anti-inflammatory and immunosuppressive properties of GCs.

The dream of such dissociated GCs may become true with a deeper understanding of how the GR is modulated. GCs trigger gene expression by either transactivation or transrepression mechanisms. There is evidence that the anti-inflammatory effects are mainly mediated by transrepression, whereas many side effects are dependent on GC-mediated transactivation. Therefore, the aim of many research groups is to identify GR ligands that preferentially induce transrepression with little transactivation activity: so-called dissociated or selective compounds. The assumption is that classical GCs influence transactivation and transrepression mechanisms to a balanced extent, whereas dissociated or selective GR ligands or modulators largely affect transrepression and have less effect on transactivation pathways.

Molecular mechanisms of GR action

Glucocorticoids exert their anti-inflammatory activity by binding to and activating the specific receptor. The GR belongs to the superfamily of nuclear receptors, is located in the cytoplasm, and functions as a ligand-activated transcription factor. Generally, two major pathways are present after the GC has bound to its receptor. The ligand–receptor complex can dimerize with a second ligand–receptor complex. Binding of this homodimer to specific DNA sequences known as glucocorticoid response elements (GRE) activates gene expression. Three types of GRE have been described so far: simple, composite, and tethering GREs, and each requires a different mechanism. The overall process is called transactivation and can lead to the biosynthesis of anti-inflammatory proteins and proteins that are responsible for side effects. Dimerisation is a prerequisite for GR binding to DNA.

In the second pathway, the ligand-activated receptor complex acts as a monomer to inhibit the transcription of target genes leading to pro-inflammatory proteins. This happens by a direct protein–protein interaction with transcription factors such as AP-1 or NF-κB. This pathway is called transrepression. As previously mentioned, the current hypothesis is that the anti-inflammatory activity of GCs is mainly triggered by transrepression, and a number of side effects are caused by transactivation mechanisms (Table 1).[4] Therefore, it is assumed that

Table 1. Mechanisms responsible for GC-mediated side effects.

Transactivation mechanisms	Transrepression mechanisms	Unknown or complex mechanisms
Diabetes mellitus	ACTH suppression	Osteoporosis
Glaucoma	Disturbed wound healing	Skin atrophy
Muscle atrophy	Infections	Hypertension
Myopathy		Cataract
		Peptic ulcer

dissociated or selective modulators, which favor the transrepression over the transactivation mechanism, should exhibit anti-inflammatory activity with fewer side effects than classical GCs.[5]

The search for dissociated GR ligands

It has been shown that it is possible to decrease inflammation in mice carrying a dimerisation-deficient GR by administra-

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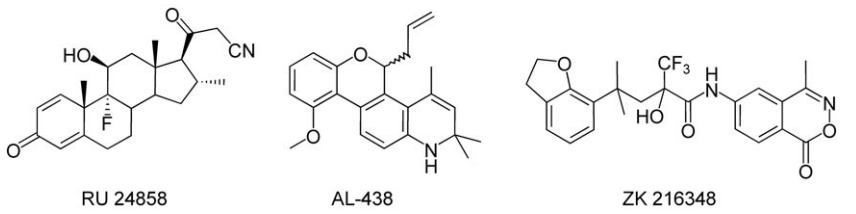
tion of dexamethasone.[6] Many companies and academic groups are conducting research programmes to identify such dissociated GR ligands. The result of the dexamethasone treatment experiment showed for the first time that the GR transrepression activity, which is intact in these animals, is sufficient for the anti-inflammatory activity of GCs. Hence, ligands that induce mainly or exclusively transrepressive activity of the GR by protein–protein interaction should display a more favourable side-effect profile than the classical GCs. A test system is necessary to identify ligands that induce stronger transrepression than transactivation activities in vitro and that attenuate inflammatory reactions at doses at which they do not induce transactivation-mediated side effects. Screening for such GR ligands commonly consists of receptor-binding assays and cellular in vitro assays to determine transrepression and transactivation activities. After a positive outcome in these in vitro assays, the compounds will be assessed in vivo to determine their potential anti-inflammatory activity and possible side effects.

Compound A, described by Haegeman and co-workers,[2] is the first example of such a dissociated structure coming from natural sources. The authors reported that this compound binds to the GR with an affinity similar to that of dexamethasone and selectively triggers transrepression but no transactivation activities, as has been proven by in vitro experiments. Compound A is as efficacious as dexamethasone in inhibiting IL-6 and E-selectin promoter activities, reflecting transrepression. Unlike dexamethasone, Compound A does not induce transactivation activity; this has been demonstrated with different test systems. Furthermore, this in vitro dissociation could be translated into the in vivo situation. According to the study, Compound A is as anti-inflammatorily active as dexamethasone in a mouse zymosan-induced paw edema model while not increasing the blood glucose level. In addition to the clear demonstration of a dissociated profile, Haegeman and colleagues performed a number of in vitro experiments to better understand the molecular mechanism of dissociation.[2] Although the described profile might be unique for Compound A, this approach may also be suitable for a more detailed investigation of the synthetic dissociated GR ligands that have been made earlier by different companies.

The first synthetic dissociated GR ligands were reported by Roussel–Uclaf (now Sanofi–Aventis): RU 24782, RU 24858, and RU 40066. The three compounds have a steroidal structure, which displays high affinity for the GR. They repress AP-1 and NF-κB activity very efficiently and display only 8–35% of the transactivation efficacy of dexamethasone. Unfortunately, these promising in vitro results could not be translated into the in vivo situation. Although RU 24858 was as anti-inflammatorily active as prednisolone in a rat asthma model, the induction of side effects (loss in body and thymus weight and the induction of osteoporosis) was observed (Figure 2).[7]

A somewhat different situation emerged with the publication of the first two nonsteroidal structures AL-438 and ZK 216348 (Figure 2). AL-438, which was synthesized through a collaboration between Abbott Laboratories and Ligand Pharmaceuticals, efficiently inhibits the production of IL-6 and E-selectin while displaying less activity in transactivation assays.[8] This advantageous behaviour was also confirmed in in vivo experiments. AL-438 is as active as prednisolone in an animal asthma model while showing a decreased potential to induce blood glucose. Furthermore, reports have been published revealing that the compound may have a lower osteoporotic potential than the classical steroid prednisolone. ZK 216348, another nonsteroidal compound, was synthesized at Schering AG.[9] The compound binds to the GR and shows a dissociated profile in vitro. It inhibits the production of IL-8 and is less active toward effects mediated by transactivation mechanisms such as the induction of tyrosine aminotransferase (TAT). TAT is one of the key enzymes involved in gluconeogenesis. This dissociated in vitro profile was also observed in vivo. ZK 216348 decreases croton oil induced ear inflammation as efficaciously as prednisolone in rats and mice following subcutaneous or topical administration. In contrast to the steroid, it displays significantly less side-effect activity such as the induction of TAT and blood glucose and decrease in body and thymus weight. Furthermore, ZK 216348 results in less skin atrophy after long-term topical treatment.

Programmes to identify GR-modulating compounds are being conducted in both pharmaceutical companies and academic research settings, including Boehringer Ingelheim, GSK, Merck Research Laboratories, Pfizer, and the University of California at San Francisco.[10]



RU 24858 AL-438 ZK 216348

Figure 2. Structures of dissociated GR ligands.

Outlook

Considerable progress has been made in identifying compounds which differentiate between transactivation and transrepression. However, there is a lot more to learn and to understand. Which side effects of the classical GCs can be avoided? Is it possible to achieve the efficacy of strong GCs without the associated side effects? How important is the recruitment of cofactors, and what role do they play?[11] Will the published X-ray crystal structure of the GR ligand-binding domain complexed with dexamethasone help to design better dissociated compounds, and will it provide more insight into the different mechanisms that affect the GR?[12] Will the dissociation found in animal experiments really translate into the human situation? Despite all these questions, which cannot be answered yet, new insight and growing knowledge

have made an old drug target very appealing again.

Keywords: glucocorticoids · immunology · inflammation · receptors · transcription

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