A NOVEL, FULLY DISSOCIATED COMPOUND OF PLANT ORIGIN FOR INFLAMMATORY GENE REPRESSION

Haegeman G*, De Bosscher K*, Vanden Berghe W*, Dewint P°, Gossye V*, Beck I*, Van Molle W†, Hennuyer‡, Hapgood J§, Libert C†, Staels B‡, Louw A§ and Elewaut D°.

*Laboratory of Eukaryotic Gene Expression and Signal Transduction (LEGEST), Department of Molecular Biology, Ghent University, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium; e-mail: guy.haegeman@ugent.be °Department of Rheumatology, University Hospital Ghent, De Pintelaan 115, Gent, Belgium. †Department for Molecular Biomedical Research, (V.I.B.) and UGent, Technologiepark 927, B-9052, Zwijnaarde, Belgium. §Department of Biochemistry, University of Stellenbosch, Matieland 7602, Stellenbosch, Rep. of South Africa. ‡Département d'Athérosclérose - U.545 Inserm Institut Pasteur de Lille, 1 rue Calmette BP245, 59019 Lille cedex, France

Summary

The identification of selective glucocorticoid receptor (GR) modifiers, which separate transactivation and transrepression properties, represents an important research goal for steroid pharmacology. Here we present that Compound A (CpdA), a plant-derived phenyl aziridine precursor, although not belonging to the steroidal class of GR-binding ligands, does mediate gene-inhibitory effects by activating GR. CpdA exerts an anti-inflammatory potential by downmodulating TNF-induced pro-inflammatory gene expression, such as IL-6 and E-selectin, but interestingly, does not enhance GRE-driven genes or induce GR binding to GRE-dependent genes *in vivo*. The anti-inflammatory mechanism involves both a reduction of the *in vivo* DNA-binding activity of p65 as well as an interference with the transactivation potential of NF-kB. Finally, CpdA is as effective as DEX in counteracting acute and chronic inflammation *in vivo,* and does not cause a hyperglycemic side effect. Taken together, this compound may be a lead compound of a novel class of anti-inflammatory agents with fully dissociated properties and might thus hold great potential for therapeutic use.

Key words: Glucocorticoid receptor (GR), Dissociative compound, Nuclear Factor kB (NF-kB), Inflammation

Introduction

NF-kB and AP-1 are eukaryotic transcription factors that play a central role in the control of different aspects of development and the immune system. An aberrant regulation can result in chronic inflammatory diseases, such as rheumatoid arthritis and bowel diseases, as well as in cancer (leukemia, bowel, prostate or breast cancer). Cytokines and adhesion molecules (IL-6, IL-8, E-selectin, ICAM, VCAM), which act as signal molecules in case of inflammatory stress, contain in their gene promoters responsive elements for the specific binding of these transcription factors (1).

Glucocorticoids (GC) or corticosteroid hormones are the most commonly used drugs to combat inflammation in the clinic. They are very effective but their long-term usage is, unfortunately, overshadowed by the occurrence of a whole range of detrimental side-effects (hyperglycemia, cataract, diabetes, muscle wasting, osteoporosis, HPA-axis suppression). This is due to the fact that endogenous GC not only play a role in immune modulation, but also in energy metabolism (sugar, carbohydrate and fat level regulation) and stress-axis regulation.

The search for GR-modulating drugs with a better side-effect profile is therefore still an actual topic of intense research. GC mediate their anti-inflammatory action through binding to the Glucocorticoid Receptor (GR), which is also a transcription factor that can regulate its target genes in the nucleus. Two ways can be distinguished: activated GR can either stimulate genes involved in glucose homeostasis by directly interacting with DNA, or else, it can repress genes involved in inflammation by protein-protein interactions with the pro-inflammatory transcription factors NF-kB and AP-1 (2). The first action mechanism is deemed responsible for the side-effects, while the latter greatly constitutes the beneficial action of GC. For this reason the pharmaceutical industry is very much interested in so-called 'dissociated ligands' of GR, that can separate both effects, do act in vivo and thus relieve at least some of the current side-effects.

Methods

ELISA, transfection of mammalian cells, reporter gene assays, immunofluorescence, EMSA, Chromatin Immunoprecipitation (ChIP), mouse models for inflammatory affection, blood analysis

Results

In this respect, we started analysing both *in vitro* and *in vivo* the promising effects of CpdA (3), characterized by us as a novel NSAID (non-steroidal anti-inflammatory

drug), and which was a stable derivative of a natural compound isolated from the Namibian desert plant, *Salsola tuberculatiformis*. Much to our surprise, this compound is able to act as a potent anti-inflammatory agent through GR, but it completely lacks transactivation properties on GRE-driven genes (4). These results not only classify CpdA as an important research tool for mechanistic studies on transactivation/transrepression mechanisms by GR, but also make CpdA an interesting drug of which we aim to study the therapeutic potential in more detail. CpdA is able to inhibit the pro-inflammatory gene expression levels of a battery of tested cytokines and adhesion molecules (IL-6, IL-8, ICAM and E-selectin), but does not mediate the gene-activating properties of GR, as assessed by testing the expression levels of the glucocorticoid-activated genes glucose-6-phosphatase and human placental alkaline phosphatase. We verified that the presence of GR is a requisite for mediating the anti-inflammatory effects of CpdA, by performing reconstitution experiments in GR-negative cells and by demonstrating a shift of GR protein from the cytoplasm to the nucleus upon CpdA treatment via indirect immunofluorescence analysis. Other class II nuclear receptors such as AR (Androgen receptor), ER (Estrogen receptor), PR (Progesterone receptor) and MR (Mineralocorticoid receptor) did not show a change in their subcellular distribution upon treatment with CpdA, which supports the receptor specificity of the compound. Through a whole cell binding assay, making use of the endogenous ligand of GR, we found that CpdA could compete for up to 80% with labelled dexamethasone for binding to GR. As for the mechanism, we found by using GR deletion mutants that both the DNA-binding domain and the ligand-binding domain are important in mediating the anti-inflammatory effects of CpdA, which is similar to the classical GCs. An important difference in the anti-inflammatory action mechanism between CpdA and glucocorticoids is that CpdA can interfere with the DNA-binding activity of NF-kB, as assayed by EMSA (Electrophoretic Mobility Shift Assay) and by the Chromatin Immunoprecipitation (ChIP) technique.

The ultimate goal is finding an anti-inflammatory compound with an improved side-effect profile, especially for chronic inflammatory disorders. Therefore, the in vivo capacity to suppress inflammation and the effect of long-term treatments was studied in the Collagen-Induced Arthritis model, a chronic inflammatory model. Clinical disease severity was assessed by scoring the number of affected joints and the degree of joint swelling. CpdA dosed at 12 mg/kg markedly reduced clinical severity compared to the PBS control group and likewise, the number of histologically affected knee joints was significantly lower in the CpdA-treated group.

As to the side-effects, administration of DEX increased glycemia compared to PBS, whereas administration of CpdA did not.

Discussion

We have characterized the anti-inflammatory (i.e. the gene-inhibitory) properties of a plant-derived product, Compound A, and were able to demonstrate its fully dissociated character *in vitro*. As this kind of products might have a better therapeutical profile, the compound was also tested in a chronic inflammatory mouse model and thus displayed its beneficial effect, without eliciting the usual and well-known side effects. This compound may thus serve as a lead compound for a novel series of anti-inflammatory drugs with a better therapeutic index.

References

1. Vanden Berghe W, De Bosscher K, Vermeulen L, De Wilde G and Haegeman G. Induction and repression of NF-κB-driven inflammatory genes. Ernst Schering Research Found Workshop, 2002; 233-278.

2. De Bosscher K, Vanden Berghe W and Haegeman G. The interplay between the Glucocorticoid Receptor and NF-κB or AP-1: Molecular mechanisms for gene repression. Endocr Rev 2003; 24: 488-522.

3. Louw A, Swart P, de Kock SS, van der Merwe KJ. Mechanism for the stabilization in vivo of the aziridine precursor -(4-acetoxyphenyl)-2-chloro-N-methyl-ethylammonium chloride by serum proteins. Biochem Pharmacol 1997; 53: 189-197.

4. De Bosscher K, Vanden Berghe W, Beck IM, Hennuyer N, Hapgood J, Van Molle W, Libert C, Staels B, Louw A & Haegeman G. A fully dissociated compound of plant origin for inflammatory gene repression. Proc Natl Acad Sci USA 2005; 102:15827-15832.