IJPSR (2012), Vol. 3, Issue 02 (Review Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 September, 2011; received in revised form 07 November, 2011; accepted 17 January, 2012

SOFT AND DISSOCIATIVE STEROIDS: A NEW APPROACH FOR THE TREATMENT OF INFLAMMATORY AIRWAY AND EYE DISEASES

Nirav P. Chandegara* and Mehul R. Chorawala

Department of Pharmacology, K. B. Institute of Pharmaceutical Education and Research, Gh-6, Sector-23, Gandhinagar-382023, Gujarat, India

Keywords:
Glucocorticoids,
Inactive metabolite approach,
Transactivation,
Transrepression

Correspondence to Author:

Nirav P. Chandegara

Department of Pharmacology, K. B. Institute of Pharmaceutical Education and Research, Gh-6, Sector-23, Gandhinagar-382023, Gujarat, India

ABSTRACT

Glucocorticosteroids (GCs) are commonly used for long-term medication in immunosuppressive and anti-inflammatory therapy, but prolonged use of GCs produce number of systemic side effects. To further improve the therapeutic index, that is the ratio of the toxic to the therapeutic dose of a drug, it is at least theoretically possible by changing both pharmacokinetics and pharmacodynamic parameters. Pharmacokinetics can deliberately be altered by using the "inactive metabolite approach" in which one can design a soft analog of a drug that is active at the site of action (e.g.,in the lung in case of inhaled medications) but undergoes a one-step predicted metabolism in the circulation and will be transformed to the very inactive metabolite from which its creation had been started. This process happens after the drug achieves its therapeutic role at the site of action and thus prevents the rest of the body to be exposed to the active drug or to various active or reactive metabolic products. Pharmacodynamic possibility to separate beneficial and deleterious effects of steroids is to try to dissociate the two main activities of glucocorticoids, which are transactivation and transrepression.

INTRODUCTION: Endogenous glucocorticoids (GC) play an essential role in maintaining body homeostasis and preventing excessive immune responses to antigenic challenges [1, 2]. Supraphysiological doses of synthetic GC are used to treat patients with inflammatory or autoimmune diseases [3].

However, the desired immunosuppressive effects of GC are accompanied by a large number of side-effects, including weight gain, diabetes, arterial hypertension, and osteoporosis. Therefore, it has been a long-standing goal of pharmacological and clinical research to identify GC that suppresses the immune system without causing such pronounced side-effects. GCs regulate carbohydrate, protein, and lipid metabolism; maintain fluid and electrolyte balance; and control cardiovascular, immune, kidney, skeletal muscle, endocrine, and nervous system functions. Inhaled corticosteroids (ICS) are considered the most effective for asthma therapy.

The ideal ICS would possess the following pharmacokinetic properties to maximize efficacy and minimize side effects: high pulmonary deposition, conversion to an active metabolite, high receptor potency, high pulmonary retention, low oral bioavailability, extensive metabolism, and rapid elimination. Inhaled corticosteroids (ICS) effectively and reproducibly repress the inflammatory processes and therefore have a central role in the treatment of asthma. They have potent and pleiotropic anti-inflammatory activity enabling downregulation of all

redundant pathways. ICS improve lung function and reduce symptoms, exacerbations, hospital readmissions, and mortality caused by asthma. ICS are considered the most effective asthma therapy and for these reasons ICS are first-line therapy for control of asthma in all patients with persistent disease. Ciclesonide is an example of a new-generation ICS. The effects of inhaled corticosteroids (ICSs) have been investigated in asthma and chronic obstructive pulmonary disease (COPD) using endobronchial biopsies. In asthma, most studies have shown reductions in infiltrating eosinophils, mast cells, and T lymphocytes. Cell associated mediators, such as cytokines derived from type 2 T-helper lymphocytes, are decreased as assessed by immunostaining and molecular biology techniques [4].

Regulation of synthesis & secretion of Glucocorticoids: Adrenocorticotropin hormone (ACTH) that is secreted from anterior pituitary controls the synthesis & release of glucocorticoids of the adrenal cortex. ACTH secretion is regulated by corticotrophin releasing hormone (CRH) which is released by CRH neurons of the endocrine hypothalamus. These three organs collectively are referred to as the hypothalamic-pituitary-adrenal (HPA) axis [1] (**fig. 1**).

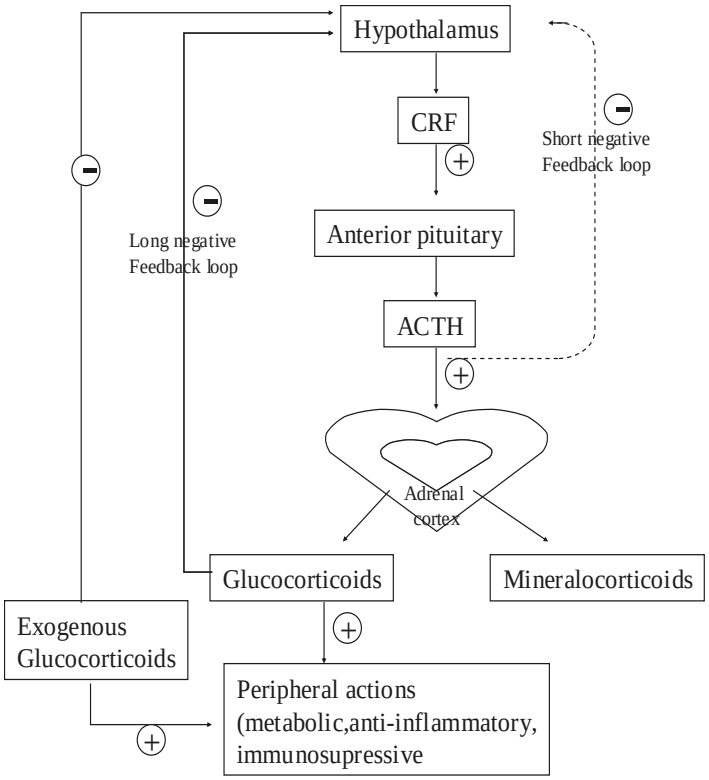

FIG. 1: REGULATION OF SYNTHESIS & SECRETION OF GLUCOCORTICOIDS

GCs inhibit ACTH secretion via direct and indirect actions on CRH neurons to decrease CRH mRNA levels and CRH release and via direct effect on corticophores. The inhibition of CRH may be mediated by specific corticosteroid receptor in hippocampus. This is called as a negative feedback of glucocorticoids.

Structure of Glucocorticoid Receptor (GR): The glucocorticoid receptor (GR) is member of the nuclear receptor super-family that includes mineralocorticoid, thyroid hormone, retinoic acid and vitamin D receptors. The GR is located at chromosome 5q31–32 and consists of nine exons, which are highly conserved across species [5]. Like all steroid receptors, the GR consists of variable N-terminal domain (regulatory domain), DNA binding domain with two zinc finger motifs, hinge region and C-terminal hormone binding domain (**Fig. 2**). The glucocorticoid receptor in its inactive state is predominantly found in the cytoplasm of target cells. It forms a complex consisting of the receptor polypeptide, two molecules of HSP90, one molecule of HSP70, and one molecule of HSP56, which is an immunophilin of the cyclosporin-, FK506- and rapamycin-binding classes. This receptor complex is stabilized by protein–protein interaction and maintains high affinity of the receptor for its ligand [6, 7].

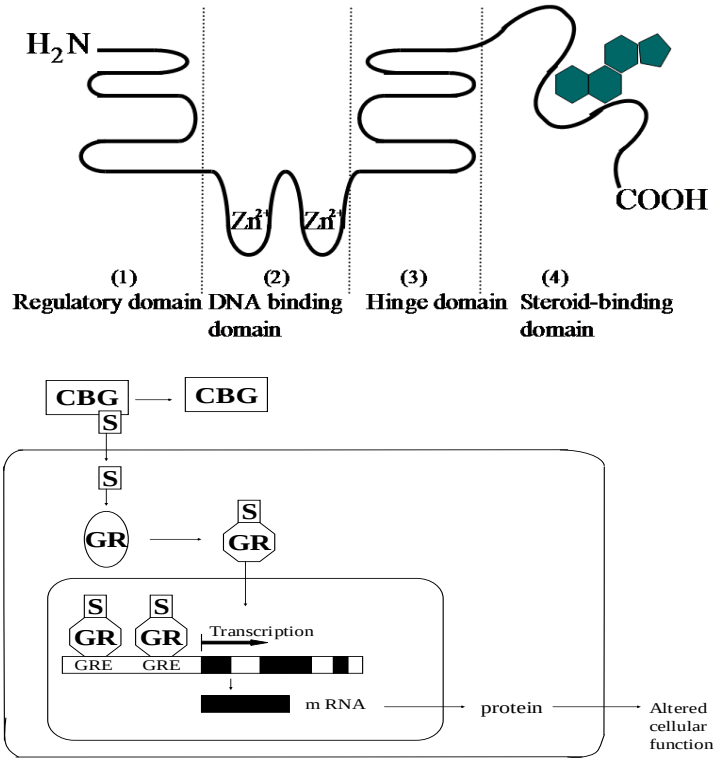

FIG. 2: (A) FUNCTION DOMAIN OF GR AND (B) INTRACELLULAR MECHANISM OF ACTION

Intracellular mechanism of action of the Glucocorticoid Receptor: Glucocorticoids, both natural (cortisol in humans, corticosterone in rodents) and synthetic (e.g. prednisolone and dexamethasone), are lipophilic and gain access to cells by diffusion across the plasma membrane. Within target cells, glucocorticoids are subject to metabolism by 11ß-hydroxysteroid dehydrogenase [8, 9]. This enzyme exists in two principal isoforms.

The type 1 enzyme acts predominantly to generate the active glucocorticoid cortisol from inactive cortisone. This enzyme is predominantly expressed in liver and adipose tissue and so acts not only to increase the circulating concentration of active glucocorticoid but can also act in a tissue-specific manner to amplify glucocorticoid action [10]. The type 2 enzyme predominantly acts in the opposite direction. This results in inactivation of cortisol by oxidation to the inactive cortisone. The tissue distribution of the type 2 enzyme is restricted to mineralocorticoid target tissues, notably the renal tubule [11, 12, 13].

The GR molecule binds glucocorticoids and transactivates or transrepresses glucocorticoid-responsive promoters (Figure 2). After binding glucocorticoid, the receptor–ligand complex undergoes a conformational change, thus releasing the HSP complex and homodimerizing with another activated GR molecule. The activated GR interacts with the importin system and translocates via the nuclear pore into the nucleus, to regulate gene expression [14, 15, 16]. In the nucleus, GR binds to glucocorticoid response elements (GREs) and subsequently recruits coactivators to the DNA in order for gene transcription to occur.

The first GREs analysed were associated with enhanced transcription; however, there are several examples of 'negative' GREs (nGREs), as described in the pro-opiomelanocortin [17], osteocalcinb [18] and prolactin promoters [19], which are associated with repression of transcription. The GR molecule acting as a monomer, in contrast, modulates the transcription rates of non-GRE-containing genes by interacting with nuclear transcription factors, including activator protein 1 (AP1), nuclear factor B (NF B) and signal transducer and activator of transcription 5 (STAT5) [20, 21] (Fig. 2). The anti-inflammatory effects are mediated to a major extent via transrepression, while many side effects are due to transactivation. Improved topical selectivity for airways and lung may be achieved if inhaled corticosteroids (ICS) were inactivated during their systemic distribution (and not just in the liver as with current ICS). Several projects have been evaluated based upon steroids inactivated by esterases. Compounds hydrolyzed by ubiquitous, nonselective esterases have failed (fluocortin butylester, itrocinonide), probably due to too rapid inactivation in the target tissue.

A new approach has been attempted based upon paraoxonase-catalyzed breakdown selectivity in plasma. This may better answer the question whether soft steroids can reach the same efficacy as current ICS in the absence of systemic activity. The GR resides in the cytoplasm complexed with several chaperones including hsp90 and immunophilin. On binding to glucocorticoids, the activated receptor dissociates from the attached accessory proteins and translocates into the nucleus.

The GR then regulates the expression of genes by several basic modes of action. From top to bottom; GR binds as a dimer to glucocorticoid response elements (GREs) in target genes to activate gene transcription; the GR binds to negative GREs (nGREs) and inhibits target gene transcription; the GR physically interacts with the c-Jun subunit of the AP-1 complex to inhibit AP-1-mediated gene expression; the GR physically interacts with the p65 (RelA) subunit of NF-_B and represses NF-_B-regulated gene expression; the GR physically interacts with members of the STAT family (STAT1, STAT5, and STAT3) and synergistically enhances STAT-regulated gene transcription [22] (**Figure 3**).

Therapeutic uses of Glucocorticoids: Bronchial asthma & other pulmonary conditions, Allergic disease, Rheumatic disorders, Replacement therapy (acute adrenal insufficiency and chronic adrenal insufficiency), Renal diseases, Infectious diseases, Ocular diseases, Skin diseases, Gastrointestinal diseases (inflammatory bowel disease), Hepatic diseases, Malignancies, Miscellaneous diseases like Organ transplantation, Spinal cord injury and Auto-immune destruction of erythrocytes.

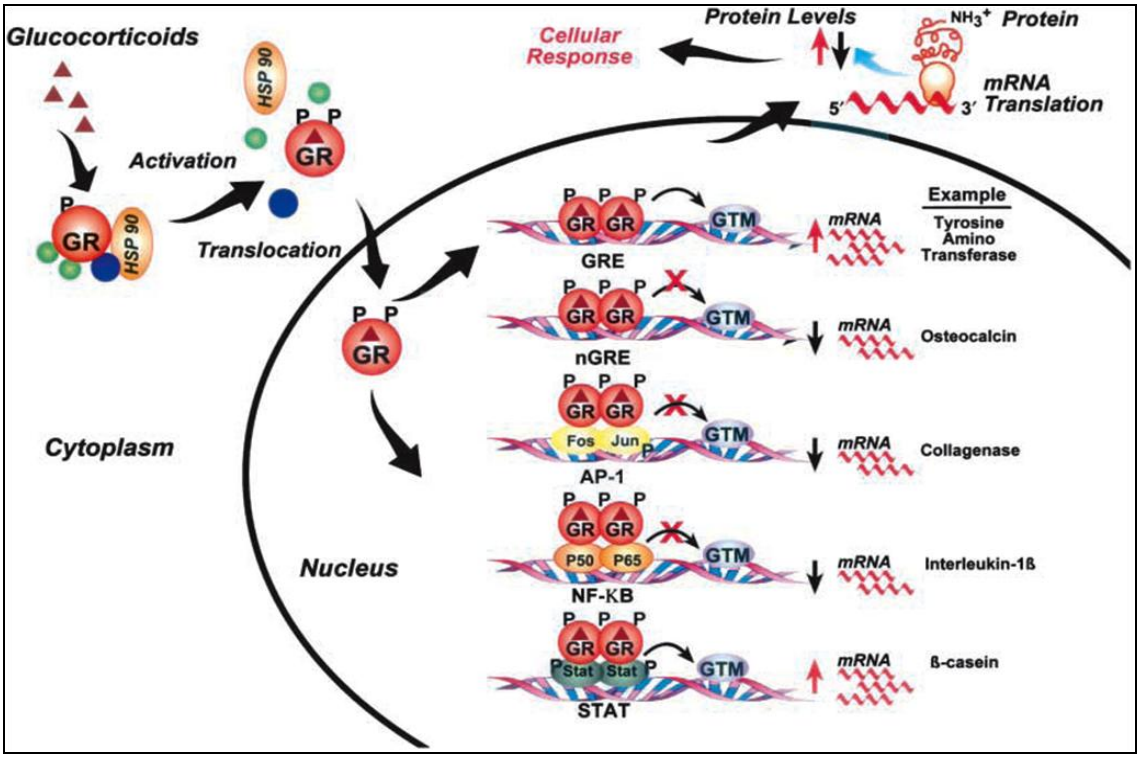

FIG. 3: BASIC MECHANISMS OF GLUCOCORTICOID RECEPTOR (GR) ACTION

Pharmacokinetic basis of airway and lung selectivity of Current Inhaled Steroids: The currently used inhaled corticosteroids (ICS) are biostable at the airways and lung target, being inactivated in liver by CYP450-3A-mediated oxidative biotransformation [23]. This brings about an efficient first-pass inactivation of the swallowed part of the inhalation dose, but not of the key fraction deposited in the airways and lung. The latter is bioavailable and transported to the heart via the bronchial and pulmonary circulations. While one quarter of the cardiac output has the first pass to the liver for inactivation, the majority of absorbed steroid is widely distributed in the body [24]. This systemic spill-over of current ICS results in circulating plasma levels of 0.1–1 nmol/l, persisting for several hours after inhalation. Although these levels are low, they are still in the same range as the KD of these very potent steroids. While this systemic spill-over introduces a risk of adverse steroid reactions [25], it does not seem to add own anti-asthmatic efficacy.

Toxicity of Glucocorticoids: Chronic uses of glucocorticoids produce following side effects:

Cushing's syndrome (buffalo hump, hypertension, euphoria, cataracts, thin arms and legs, increased abdominal fat), Hyperglycemia, Increased susceptibility to infection and risk for reactivation of latent tuberculosis, Osteoporosis, Osteonecrosis, Steroid myopathy, Cataracts, Behavioral changes and Risk of peptic ulcers. Therapeutic index can be improved by changing pharmacokinetics and pharmacodynamic parameters.

Pharmacokinetics can be altered by using the "inactive metabolite approach" [26, 27, 28] in which one can design a soft analog of a drug that is active at the site of action but undergoes a one-step predicted metabolism in the circulation and will be transformed to the inactive metabolite from which its creation had been started [29]. This process happens after the drug achieves its therapeutic role at the site of action and thus prevents the rest of the body to be exposed to the active drug or to various active or reactive metabolic products.

Pharmacodynamic possibility to separate beneficial and deleterious effects of steroids is to try to dissociate the two main activities of glucocorticoids, which are transactivation and transrepression. However, it has been shown recently that by mutating individual amino acids in different domains of the GR transactivation and transrepression became two separable functions [30] and studies with synthetic glucocorticoid derivatives have proved that it is possible to dissociate these two properties of the steroid molecule [31].

What is a Soft Steroid? A soft drug is active by itself, has therapeutic efficacy at the site of application and is rapidly inactivated during its systemic uptake and distribution. A soft ICS should have sufficient metabolic stability for inducing the desired anti inflammatory effect at the airways and the lung target & inactivated during its systemic uptake and distribution [32]. Various types of soft steroids are:

First-generation Cortienic Acid-Based Soft Steroids: Loteprednol etabonate and analogues. Loteprednol etabonate (LE) is an active corticosteroid that lacks serious side effects and has been approved by the Food and Drug Administration (FDA) as the active ingredient of 3 ophthalmic preparations (Lotemax, Alrex, Zylet) [33, 34].

At present, it is the only corticosteroid approved by the FDA for use in all inflammatory and allergy-related ophthalmic disorders, including inflammation after cataract surgery, uveitis, allergic conjunctivitis, and giant papillary conjunctivitis (GPC). LE resulted from a classic inactive metabolite-based SD approach that used cortienic acid as starting point (Figure 3) [35-42].

Hydrocortisone is known to undergo a variety of oxidative and reductive metabolic conversions [43]. Oxidation of its dihydroxyacetone side chain leads to formation of cortienic acid through a 21-aldehyde (21-dehydrocortisol) and a 21-acid (cortisolic acid). Cortienic acid is an ideal lead for the inactive metabolite approach because it lacks corticosteroid activity and is a major metabolite excreted in human urine. To obtain new active soft compounds, the pharmacophore moieties of the 17α and β side chains have to be restored by suitable isosteric/isoelectronic substitution containing esters or other types of functions that restore the original corticosteroid activity and also incorporate hydrolytic features to ensure adequate metabolic properties.

Modifications of the 17β ester function and the 17α hydroxy function, together with other changes (e.g., introduction of fluorination at 6α and/or 9α, methylation at 16α or 16β), led to a host of analogs representing the first generation of cortienic acid-based soft steroids (**Figure 4**). More than 120 of these soft steroids have been synthesized starting in the late 1970s and during a systematic synthetic study performed in collaboration with Otsuka Pharmaceutical Co (Tokyo, Japan) [44, 45, 46]. Critical functions for activity are a haloester in the 17β position and a novel carbonate [47, 48] or ether [49] substitution in the 17α position.

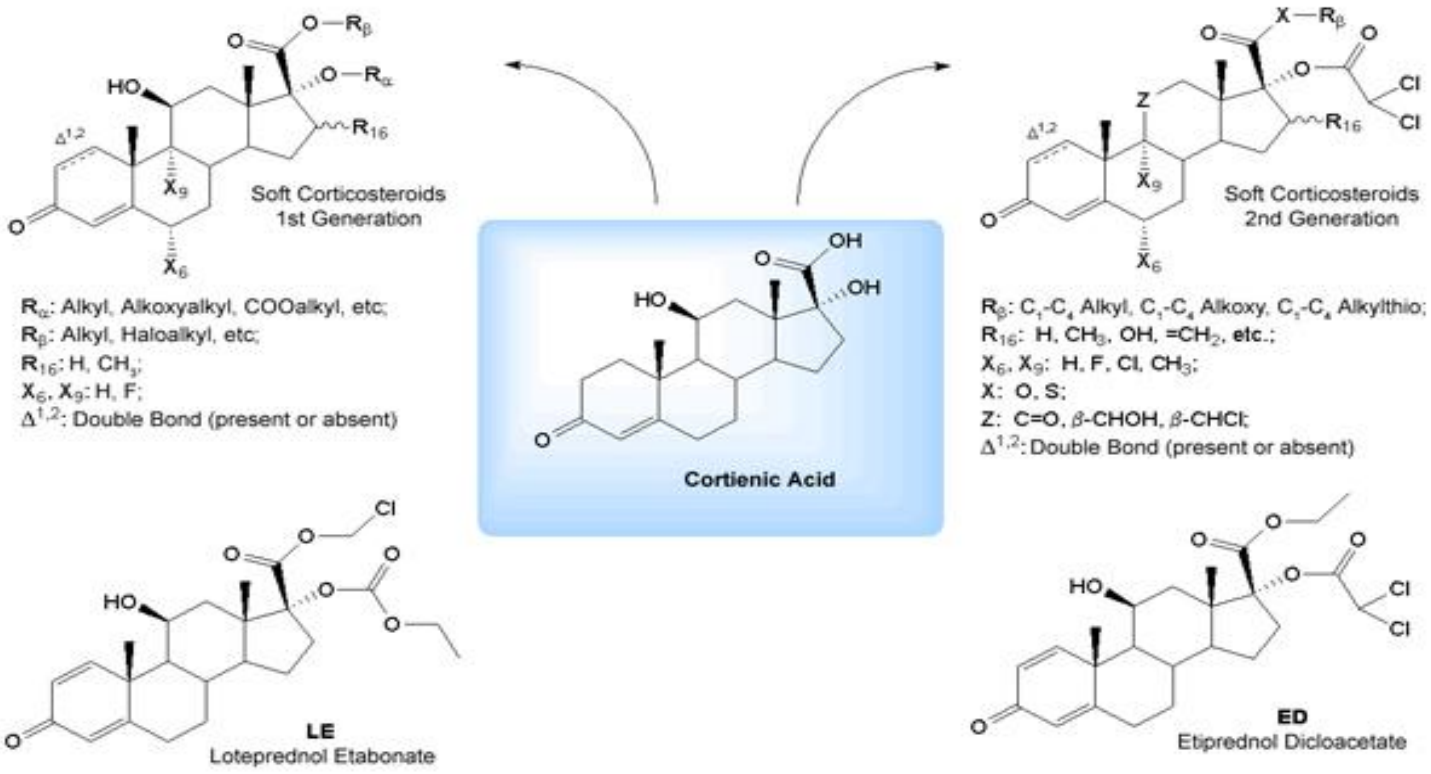

FIG. 4: DESIGN OF FIRST- AND SECOND-GENERATION CORTIENIC ACID-BASED SOFT STEROIDS

LE is indeed active and is metabolized into its predicted metabolites (Figure 4), and these metabolites are inactive [50]. The pharmacokinetic profile of LE indicates that, when absorbed systemically, it is rapidly transformed to the inactive 17β-carboxylic acid metabolite and eliminated from the body mainly through the bile and urine [51, 52, 53]. LE did not affect IOP in rabbits [54], and later various human studies [55] also confirmed that it has no effect on IOP. Consistent with the soft nature of this steroid, systemic levels or effects cannot be detected even after chronic ocular administration [56].

Second-generation Cortienic Acid- Based Soft Steroids: Etiprednol Dicloacetate and Analogs. More recently, a new class of soft steroids with 17α-dichloroester substituent has been identified (**Figure 5**) [57]. This is a unique design that no known corticosteroid contains halogen substituents at the 17α position. Nevertheless, the pharmacophore portions of these second-generation cortienic acid-based soft steroids, including the halogen atoms at 17α, can be positioned so as to provide excellent overlap with those of the traditional corticosteroids [58].

Dichlorinated substituents seem required for activity and sufficiently soft nature, and justifications seem likely. First, with dichlorinated substituents, one of the chlorine atoms will necessarily point in the direction needed for pharmacophore overlap, but with monochlorinated substituents, steric hindrance will force the lone chlorine atom to point away from this desired direction. Second, whereas compared with the unsubstituted ester, dichloro substituents cause an ~20-fold increase in the second-order rate constant k_{cat}/K_M of enzymatic hydrolysis in acetate esters, monochloro substituents do not cause any change [59].

Contrary to the first generation of soft steroids, in this second generation, hydrolysis primarily cleaves not the 17β-, but the 17α-positioned ester. Nevertheless, the corresponding metabolites are also inactive. From this series, etiprednol dicloacetate (ED, Figure 4) was selected for development. ED has shown better receptor binding affinity (RBA) than LE and was proven as or even more effective than budesonide in various asthma models. In agreement with its soft nature, ED was found to have low toxicity in animal models and in human clinical trials [60, 61, 62].

The no observable adverse effect level (NOAEL) of ED after 28-day oral administration was found to be 2 mg/kg in rats and dogs, ~40 times higher than that of budesonide [63].

FIG. 5: METABOLISM OF LE

Advantages over ICS: More therapeutic index, devoid of systemic side effects, does not increase intra-ocular pressure.

Therapeutic uses: Bronchial asthma, Inflammation in eye (Blepharitis, Giant papillary conjunctivitis, Cataract surgery) and Postoperative inflammation.

Novel Corticosteroids: Corticosteroids produce their effects by activating the glucocorticoid receptor in cells to directly or indirectly regulate transcription of target genes. The principal molecular mechanisms by which corticosteroids modify gene expression are transactivation (positive regulation of gene transcription) and transrepression (negative regulation of gene transcription). The anti-inflammatory effects of corticosteroids are mediated to a major extent via transrepression, while many side effects are due to transactivation. A new generation of corticosteroids is being developed that preferentially induce transrepression with little or no transactivation. These drugs are becoming known as “dissociated” steroids

because of the dissociation between transrepression and transactivation. Another new approach is to develop a "soft" steroid, one that has limited or no systemic side effects because it is delivered only close to its site of action or is degraded into inactive metabolites. The most promising agent for asthma is ciclesonide, a prodrug that is cleaved by esterase into an active form in the lung. The active compound is without clinically relevant effects on the hypothalamic-pituitary-adrenal axis, because it is activated only in bronchial mucosa and the absorbed fraction is highly plasma protein–bound. In patients with mild allergic asthma, Larsen and colleagues determined that ciclesonide significantly reduces the decline in FEV1 after antigen challenge, from 0.426 to 0.233 L soon after the challenge and from 0.44 to 0.213 L in the late phase.

Postma and colleagues have documented that ciclesonide is equally effective whether inhaled in the morning or evening, although evening administration seems to lead to more pronounced improvement in morning PEFR. The currently used inhaled corticosteroids (ICS) are biostable at the airways and lung target, being inactivated in liver by CYP450-3A-mediated oxidative biotransformation. This brings about an efficient first-pass inactivation of the swallowed part of the inhalation dose, but not of the key fraction deposited in the airways and lung. The latter is bioavailable and transported to the heart via the bronchial and pulmonary circulations. While one quarter of the cardiac output has the first pass to the liver for inactivation, the majority of absorbed steroid is widely distributed in the body [64].

What is Dissociative Glucocorticoid? GCs, which are able to dissociate transactivation and transrepression of certain target genes, which leads to separation of therapeutic effects from side effects. Endogenous glucocorticoids (GC) play an essential role in maintaining body homeostasis and preventing excessive immune responses to antigenic challenges [1, 2]. Supraphysiological doses of synthetic GC are used to treat patients with inflammatory or autoimmune diseases [3]. However, the desired immunosuppressive effects of GC are accompanied by a large number of side-effects, including weight gain, diabetes, arterial hypertension, and osteoporosis.

Therefore, it has been a long-standing goal of pharmacological and clinical research to identify GC that suppresses the immune system without causing such pronounced side-effects.

At the molecular level, the effects of GCs are mediated by the intracellular glucocorticoid receptor (GR). GR is a ligand-dependent transcription factor, which upon hormone binding, translocates to the cell nucleus, where it binds to glucocorticoid response elements (GREs) in the promoter regions of target genes, resulting in *trans*-activation of these genes [65-69]. *Trans*-activation is probably the predominant mechanism by which GCs exert many of their metabolic and cardiovascular side-effects [71-73].

In contrast, the anti-inflammatory/immunosuppressive effects of GCs involve *trans*-repression of target genes not containing any GR-binding sites [2, 3, 74, 75]. The human interleukin-2 (IL-2) gene is the prototype of a key immune gene that is repressed by GC. GC-mediated repression of IL-2 gene expression is thought to be due to direct interaction of GR with other transcriptional enhancers, such as activating protein-1 (AP-1) and nuclear factor-kB (NF-kB) [59-65].

Conventional GCs do not dissociate *trans*-activation and *trans*-repression. Strategies to develop improved GCs aim to maintain *trans*-repression of immune genes in the absence of significant *trans*-activation of GRE-dependent promoters. Compared to conventional glucocorticoids, Medroxyprogesterone acetate (MPA) can be reffered to as a dissociative glucocorticoid, its transrepression/transactivation ratio being 6.6 (transrepression 1.91/transactivation 0.29), with dexamethasone being the standard (transrepression 1/transactivation 1).

Based on this, we can conclude that MPA is a highly promising substance for the treatment of autoimmune/inflammatory diseases. Dissociated steroids such as RU24858 to be almost as effective as dexamethasone in inducing transrepression but show little or no transactivation ability in human and murine cell lines. Nonsteroidal selective GR-agonist, ZK 216348 shows a significant dissociation between transrepression and transactivation both *in vitro* & *in vivo* [68].

REFERENCES:

1. Chrousos GP: The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N Engl J Med 1995; 332:1351–1362.
2. Wick G, Hu Y, Schwarz S, Kroemer G. Immunoendocrine communication via the hypothalamo-pituitary-adrenal axis in autoimmune disease. Endocr Rev 1993; 14:539–563.
3. Boumpas DT, Paliogianni F, Anastassiou ED, Balow JE. Glucocorticosteroid action on the immune system: molecular and cellular aspects. Clin Exp Rheumatol 1991; 9:413–423.
4. Wardlaw AJ, Brightling CE, Green R et al. New insights into the relationship between airway inflammation and asthma Clin Sci 2002; 103:201–211.
5. Stolte EH, van Kemenade BM, Savelkoul HF & Flik G. Evolution of glucocorticoid receptors with different glucocorticoid sensitivity. J Endocrinol 2006; 190:17–28.
6. Galigniana MD, Housley PR, DeFranco DB & Pratt WB. Inhibition of glucocorticoid receptor nucleocytoplasmic shuttling by okadaic acid requires intact cytoskeleton. J Biol Chem 1999; 274:16222–16227.
7. Galigniana MD, Radanyi C, Renoir JM, Housley PR & Pratt WB. Evidence that the peptidylprolyl isomerase domain of the hsp90-binding immunophilin FKBP52 is involved in both dynein interaction and glucocorticoid receptor movement to the nucleus. J Biol Chem2001; 276:14884–14889.
8. Seckl JR.11ß-Hydroxysteroid dehydrogenases: changing glucocorticoid action. Curr Opin Pharmacol 2004; 4:597–602.
9. Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, Hewison M & Stewart PM. 11ß-Hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. Endocr Rev 2004; 25:831–866.
10. Stewart PM. Tissue-specific Cushing's syndrome, 11ß-hydroxysteroid dehydrogenases and the redefinition of corticosteroid hormone action. Eur J Endocrinol 2003;149:163–168
11. Holmes MC, Sangra M, French KL, Whittle IR, Paterson J, Mullins JJ & Seckl JR. 11ß-Hydroxysteroid dehydrogenase type 2 protects the neonatal cerebellum from deleterious effects of glucocorticoids. Neuroscience 2006; 137:865–873.
12. Paterson JM, Seckl JR & Mullins JJ. Genetic manipulation of 11ß-hydroxysteroid dehydrogenases in mice. Am J Physiol Regul Integr Comp Physiol 2005; 289:R642–R652.
13. Seckl JR, Yau J & Holmes M. 11ß-Hydroxysteroid dehydrogenases: a novel control of glucocorticoid action in the brain. Endocr Res 2002; 8:701–707.
14. Hager GL. The dynamics of intranuclear movement and chromatin remodeling by the glucocorticoid receptor. Ernst Schering Res Found Workshop 2002; 40:111–129.
15. Elbi C, Walker DA, Romero G, Sullivan WP, Toft DO, Hager GL & DeFranco DB. Molecular chaperones function as steroid receptor nuclear mobility factors. Proc Natl Acad Sci USA 2004; 101:2876–2881.
16. Nagaich AK, Walker DA, Wolford R & Hager GL. Rapid periodic binding and displacement of the glucocorticoid receptor during chromatin remodeling. Mol Cell 2004; 14:163–174.
17. Drouin J, Sun YL, Chamberland M, De Gauthier YLA, Nemer M & Schmidt TJ. Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. EMBO J 1993; 12:145–156.
18. Meyer T, Gustafsson JA & Carlstedt-Duke J. Glucocorticoid-dependent transcriptional repression of the osteocalcin gene by competitive binding at the TATA box. DNA Cell Biol 1997; 16:919–927.
19. Sakai DD, Helms S, Carlstedt-Duke J, Gustafsson JA, Rottman FM & Yamamoto KR. Hormone-mediated repression: a negative glucocorticoid response element from the bovine prolactin gene. Genes Dev 1998; 2:1144–1154.
20. Rogatsky I, Luecke HF, Leitman DC & Yamamoto KR. Alternate surfaces of transcriptional coregulator GRIP1 function in different glucocorticoid receptor activation and repression contexts. Proc Natl Acad. Sci U S A 2002; 99:16701–16706.
21. Rogatsky I, Wang JC, Derynck MK, Nonaka DF, Khodabakhsh DB, Haqq CM, Darimont BD, Garabedian MJ & Yamamoto KR. Target-specific utilization of transcriptional regulatory surfaces by the glucocorticoid receptor. Proc Natl Acad Sci U S A 2003; 100:13845–13850.
22. Bousquet J, Jeffery PK, Busse WW et al. Asthma: from bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med 2000; 161:1720–1745.
23. Hansel TT, Barnes PJ. New Drugs for Asthma, Allergy and COPD. Prog Respir Res. 2001; 31:94–97.
24. Bodor N .Designing safer drugs based on the soft drug approach. Trends Pharmacol Sci 1982; 3:53–56.
25. Bodor N. Soft drugs: principles and methods for the design of safer drugs. Med Res Rev 1984; 3:449–469.
26. Bodor N: Soft drugs, in Encyclopedia of Human Biology (Dulbecco R ed).Academic Press,San Diego,vol 7,1991:1–27.
27. Bodor N. Recent advances in retrometabolic drug design approaches. J ContrRelease 1999; 62:209–222.
28. Heck S, Kullmann M, Gast A, Ponta H, Rahmsdorf HJ, Herrlich P, and Cato ACB.A distinct modulating domain in glucocorticoids receptor monomers in the repression activity of the transcription factor AP-1.Eu Mol Biol Organ 1994;13:4087–4095.
29. Vayssiere BM, Dupont S, Choquart A, Petit F, Garcia T, Marchandeau C, Gronemeyer H, and Resche-Rigon M. Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepression exhibit anti-inflammatory activity in vivo. Mol Endocrinol 1997; 11:1245–1255.
30. Bodor N, Buchwald P. Soft drug design. General principles and recent applications. Med Res Rev 2000; 20:58-101.
31. Noble S, Goa KL. Loteprednol etabonate: clinical potential in the management of ocular inflammation. BioDrugs 1998; 10:329-339.
32. Howes JF. Loteprednol etabonate: a review of ophthalmic clinical studies. Pharmazie 2000; 55:178-183.
33. Bodor N, Buchwald P. Design and development of a soft corticosteroid, loteprednol etabonate. In: Schleimer RP, O'Byrne PM, Szefler SJ, Brattsand R. Inhaled Steroids in Asthma. Optimizing Effects in the Airways. New York, Marcel Dekker 2002:541-564.
34. Bodor N. Steroids having anti-inflammatory activity. Belgian patent BE889, 563 (Internat Classif C07J/A61K); 3:1981.
35. Bodor N, Varga M. Effect of a novel soft steroid on the wound healing of rabbit cornea. Exp Eye Res 1990; 50:183-187.
36. Druzgala P, Hochhaus G, Bodor N. Soft drugs: Blanching activity and receptor binding affinity of a new type of glucocorticoid: loteprednol etabonate. J Steroid Biochem 1991; 38:149-154.
37. Bodor N, Loftsson T, Wu W-M. Metabolism, distribution, and transdermal permeability of a soft corticosteroid, loteprednol etabonate. Pharm Res 1992; 9:1275-1278.
38. Hochhaus G, Chen L-S, Ratka A *et al.* Pharmacokinetic characterization and tissue distribution of the new glucocorticoid soft drug loteprednol etabonate in rats and dogs. J Pharm Sci 1992; 81:1210-1215.
39. Bodor N, Murakami T, Wu W-M. Soft drugs:Oral and rectal delivery of loteprednol etabonate, a novel soft corticosteroid, in rats - for safer treatment of gastrointestinal inflammation. Pharm Res 1995; 12:869-874.

40. Bodor N, Wu W-M, Murakami T, Engel S. Soft drugs: Pharmacokinetics, metabolism and excretion of a novel soft corticosteroid, loteprednol etabonate, in rats. Pharm Res 1995; 12:875-879.
41. Monder C, Bradlow HL. Cortoic acids: explorations at the frontier of corticosteroid metabolism. Recent Prog Horm Res 1980; 36:345-400.
42. Bodor N. Novel approaches for the design of membrane transport properties of drugs. In: Roche EB, ed. Design of Biopharmaceutical Properties through Prodrugs and Analogs. Washington, DC: Academy of Pharmaceutical Sciences; 1977:98-135.
43. Bodor N. Designing safer drugs based on the soft drug approach. Trends Pharmacol Sci 1982; 3:53-56.
44. Bodor N. Soft drugs: strategies for design of safer drugs. Metabolisme et Conception Medicaments: Quo Vadis? Proceedings of Symposium at Montpellier, France; November 26-27, 1981; Montpellier, France: CLIN MIDY; 1983: 217-251.
45. Druzgala P, Bodor N. Regioselective O-alkylation of cortienic acid and synthesis of a new class of glucocorticoids containing a 17α-alkoxy, a 17α-(1'-alkoxyethyloxy), a 17α-alkoxymethyloxy, or a 17α-methylthiomethyloxy function. Steroids 1991; 56:490-494.
46. Bodor N, Bodor N, Wu W-M. A comparison of intraocular pressure elevating activity of loteprednol etabonate and dexamethasone in rabbits. Curr Eye Res 1992; 11:525-530.
47. Novack GD, Howes J, Crockett RS, Sherwood MB. Change in intraocular pressure during long-term use of loteprednol etabonate. J Glaucoma 1998; 7:266-269.
48. Howes J, Novack GD. Failure to detect systemic levels and effects of loteprednol etabonate and its metabolite, PJ-91, following chronic ocular administration. J Ocul Pharmacol Ther 1998; 14:153-158.
49. Ilyas H, Slonim CB, Braswell GR, Favetta JR, Schulman M. Long-term safety of loteprednol etabonate 0.2% in the treatment of seasonal and perennial allergic.
50. Bodor N. Androstene derivatives. US patent 5 981 517. November 9, 1999.
51. Barton P, Laws AP, Page MI. Structure-activity relationships in the esterase-catalysed hydrolysis and transesterification of esters and lactones. J Chem Soc 1994; 2021-2029.
52. Kurucz I, Tóth S, Németh K et al. Potency and specificity of the pharmacological action of a new, antiasthmatic, topically administered soft steroid, etiprednol dicloacetate (BNP-166). J Pharmacol Exp Ther 2003; 307:83-92.
53. Kurucz I, Németh K, Mészáros S et al. Anti-inflammatory effect and soft properties of etiprednol dicloacetate (BNP-166), a new, anti-asthmatic steroid. Pharmazie 2004; 59:412-416.
54. Evans RM. The steroid and thyroid hormone receptor superfamily. Science 1988; 240:889–895.
55. Truss M, Beato M. Steroid hormone receptors: interaction with deoxyribonucleic acid and transcription factors. Endocr Rev 1993; 14:459–479.
56. Tsai M-J, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 1994; 63:451–486.
57. Beato M, Herrlich P, Schütz G. Steroid hormone receptors: many actors in search of a plot. Cell 1995; 83:851–857.
58. Mangelsdorf DJ, Thummel C, Beato M *et al*. The nuclear receptor superfamily: the second decade. Cell 1995; 83:835–839.
59. Bamberger CM, Schulte HM, Chrousos GP. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr Rev 1996; 17:245–261.
60. Becker PB, Gloss B, Schmid W, Strähle U, Schütz G. In vivo protein-DNA interactions in a glucocorticoid response element require the presence of the hormone. Nature 1986; 324:686–688.
61. Imai E, Miner JN, Mitchell JA, Yamamoto KR, Granner DK. Glucocorticoid receptor-cAMP response element-binding protein interaction and the response of the phosphoenolpyruvate carboxykinase gene to glucocorticoids. J Biol Chem 1993; 268:5353–5356.
62. Brasier AR, Li J. Mechanisms for inducible control of angiotensinogen gene transcription. Hypertension 1996; 27:465–475.
63. Bamberger A-M, Erdmann I, Bamberger CM, Jenatschke S, Schulte HM. Transcriptional regulation of the human leukemia inhibitory factor gene: modulation by glucocorticoids and estradiol. Mol Cell Endocrinol 1997; 127:71–79.
64. Georgitis JW. The 1997 asthma management guidelines and therapeutic issues relating to the treatment of asthma. Chest 1999; 115:210–217.
65. Northrop JP, Crabtree GR, Mattila PS. Negative regulation of interleukin 2 transcription by the glucocorticoid receptor. J Exp Med 1992; 175:1235–1245.
66. Vacca A, Felli MP, Farina AR, et al. Glucocorticoid receptor-mediated suppression of the interleukin 2 gene expression through impairment of the cooperativity between nuclear factor of activated T cells and AP-1 enhancer elements. J Exp Med 1992; 175:637–646.
67. Paliogianni F, Raptis A, Ahuja SS, Najjar SM, Boumpas DT. Negative transcriptional regulation of human interleukin 2 (IL-2) gene by glucocorticoids through interference with nuclear transciption factors AP-1 and NF-AT. J Clin Invest 1993; 91:1481–1489.
68. Jonat C, Rahmsdorf HJ, Park KK, Cato AC, Gebel S, Ponta H, Herrlich P. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell 1990; 62:1189–1204.
69. Schüle R, Rangarajan P, Kliewer S et al. Functional antagonism between oncoprotein c-jun and the glucocorticoid receptor. Cell 1990; 62:1217–1226.
70. Yang-Yen H-F, Chambard JC, Sun YL, Smeal T, Schmidt TJ, Drouin J, Karin M. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. Cell 1990; 62:1205–1215.
71. Scheinman RI, Gualberto A, Jewell CM, Cidlowski JA, Baldwin ASJ. Characterization of mechanisms involved in transrepression of NF-kB by activated glucocorticoid receptors. Mol Cell Biol 1995; 15:943–953.
72. Bamberger CM, Schulte HM. Molecular mechanisms of dissociative glucocorticoid activity. European Journal of Clinical Investigation 2000; 30:6–9.
73. Vayssiere BM, Dupont S, Choquart A, Petit F, Garcia T, Marchandeau C, Gronemeyer H, Resche-Rigon M: Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepression exhibit anti-inflammatory activity in vivo. Mol Endocrinol 1997, 11:1245-1255.
74. Vanden Berghe W, Francesconi E, De Bosscher K, Resche-Rigon M, Haegeman G: Dissociated glucocorticoids with anti-inflammatory potential repress interleukin-6 gene expression by a nuclear factor-kappa B-dependent mechanism. Mol Pharmacol 1999; 56:797-806.
75. Schäcke H, Schottelius A, Döcke WD, Strehlke P, Jaroch S.Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. Proceedings of the National Academy of Science; 101(1):227-232.