# Clinical Potencies of Glucocorticoids: What do we Really Measure?

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**Abstract:** Glucocorticoids (GC) are used in pulmonary medicine since the early nineteen-thirties; in the beginning by using extracts of adrenal glands of animals, later on synthetically composed and since the early nineteen-seventies in inhaled formulation. In pulmonary medicine the majority of prescriptions are related to asthma and exacerbations of COPD. In determining the pharmacological potency of the different GC's many efforts were made for quantification. In this respect *in vitro, in vivo, ex vivo* and clinical studies were performed. Examples in estimating the GC potency range from skin-blanching tests to suppressive effect on the adrenal gland, the latter representing 'a classical paradigm'. Thus far different quantifying attempts that have been made did not take into account the tissue concentration-effect relationship, which can be achieved by pharmacokinetic/pharmaco-dynamic (PK/PD) modelling. Moreover, in the clinical studies described, the suggestion was risen that GC's may have a different potency towards each target tissue separately and that the suppressive effect on the adrenal gland does not reflect for instance its anti-inflammatory action. In this respect the studies describe a quantifying inflammation model for asthma e.g. by granulocyte colony stimulating factor (GCSF) stimulated rise in eosinophilic cationic protein (ECP), which could be inhibited by different GC's.

In conclusion, studies on potencies of GC should comprise PK/PD modelling and should target as much as possible on the different outcome parameters of the therapeutical and adverse effects separately and simultaneously, thereby describing 'the spectrum of potency' of a GC rather than 'the potency'.

## INTRODUCTION

### Historical Milestones

In the nineteen thirties, researchers discovered the effects of hormones produced in the cortex of the adrenal gland. Extracts were firstly isolated in 1927 by the groups of Hartman and Mc Arthur, and Rogoff and Stewart in cats and dogs [1, 2]. In 1932 Fineman described the use of this cortisol containing extract in the clinical management of bronchial asthma [3]. After the second world war, physicians started treatments with ACTH, later on followed by purified cortisone [45]. From 1951, various aerosolized glucocorticoid (GC) formulations were introduced, but with undesired systemic adverse effects. In 1972 Morrow, Brown, *et al*. described the selective GC aerosol formulation beclomethasone dipropionate, with less systemic side effects when administered in a locally appropriate dose [6]. GC's both inhaled and systemically administered, are at present well accepted as one of the cornerstones of the treatments for asthma and exacerbations of Chronic Obstructive Pulmonary Disease (COPD).

### Genomic and Non Genomic Mechanisms of Glucocorticoids (GC's)

Along with many other effects, GC's encompass anti-inflammatory properties probably related to effects towards micro-vasculature and cells [7-9]. GC's alter biological behaviour of T-lymphocytes involved in inflammatory cascades. Corticosteroids (CS) in general exhibit their pharmacological effects via genomic and non-genomic mechanisms. In accordance with the hypothesis of the genomic mechanism, lipophilic GC's are believed to cross the cell membrane and to bind to the cytosolic GC-receptor, after which event two "heat shock" proteins are released. The GC-receptor-complex then passes through the nuclear membrane and binds to the glucocorticoid-responsive-elements (GRE's) of the genomic DNA, followed by interaction with a nuclear transcription factor [10-12]. The duration of the aforementioned processes varies from 30 minutes to several hours. However, a number of pharmacological responses on GC's and other CS's are observed within seconds to minutes, which exclude genomic action and is therefore referred to as a nongenomic action. Non-genomic mechanisms are believed to take place through two modes of actions: interaction of CS's with specific receptors or with non-specific proteins and/or membrane lipids. In addition, interactions have also been described between genomic and nongenomic mechanisms, thereby controlling genomic mechanisms through nongenomic mechanisms [13].

### Influences of GC's on Inflammatory Mediators in Asthma

#### *Cytokines*

Many mediators of inflammation have been recognised to be involved in the pathophysiology of different inflammatory diseases involving the lung. $Th_2$ lymphocyte expression, characterized by cytokine production appears to be predominantly present in asthma. To illustrate, cytokines

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are cellular proteins, involved in the regulation of cell function. The typical cytokine is a glycosylated protein, transiently secreted by a stimulated effector cell. Cytokine effects originate from binding to specific receptors on target tissues [14]. GC's inhibit cytokine production of cells involved in the inflammatory cascade of asthma.

### *Leukotrienes*

Leukotrienes (cystenyl leukotrienes) are another series of peptides that are considered as inflammatory mediators in asthma. In 1938, Feldberg and Kellaway discovered a biological activity generated by the interaction between cobra venom and guinea pig lung tissue [15]. This biological activity was described initially as slow reacting substance of anaphylaxis (SRS-A), and was characterized later on in 1979 as: SRS-A = $LTC_4$ + $LTD_4$ + $LTE_4$ [16].

GC's inhibit the eosinophilic granulocyte in the production of $LTC_4$ and PAF, through inhibition of of $IL_3$, $IL_5$ and GM-CSF production by T-lymfocytes, macrophages and endothelial cells [17]. Direct inhibition by GC's regarding arachidonic acid cascade has not been clearly demonstrated [18, 19].

## Current Views on the Clinical Potencies of Glucocorticoids

### *Historical Concepts*

Clinical potencies of GC's and mineralocorticoids (MC) have been difficult to assess although classical approaches were accepted in clinical practice. The citation in Table **1.1** is an example of one of the historical concepts, extracted from one of the various textbooks [20]. Table **1.1** shows the difference between GC's and MC's and suggests that clinical effectiveness of a GC is reflected by its potency to suppress cortisol production. By its differentiation of CS's in GC and MC, already then the suggestion is made that different CS's may have a different potency per target, however within the classification of GC or MC this difference in potency per target was not addressed.

## ASSESSMENT OF POTENCIES USING VARIOUS METHODS

The potency of systemic effects of GC's is frequently expressed as the ability to suppress cortisol production. Apart from this, another effect of GC's, i.e. the suppression of inflammation, is not easily quantified. In clinical studies anti inflammatory efficacy of GC's towards several target tissues and cells have been substituted by clinical outcome while laboratory studies, focussing at the potencies of GC's, were expressed as influences on cellular biological processes and surrogate markers. A few examples are discussed below.

## *In Vitro* Studies

### *Inhibition of PHA Stimulated Lymphocytes*

Cantrill *et al*. published a paper in 1975 were the potencies of three corticosteroids were compared [21]. CS

**Table 1.1. Characteristics of Some Adrenocorticosteroid Preparations**

| Drug | Estimated potencies[1] | | Equivalent 'anti-inflammatory' 'effectiveness' (per nearest whole tablet) | Daily Dose above which HPA axis suppression possible[2] (mg) | | Approx. plasma half-life (min) | Biological half-life (hrs) |
|---|---|---|---|---|---|---|---|
| | Gluco-corticoid | Mineralo-corticoid | | male | female | | |
| Cortisol (hydrocortisone) | 1 | 1 | 20 mg | 20-30 | 15-25 | 90 | 8-12 |
| Cortisone | 0.8 | 0.8 | 25 mg | 25-35 | 20-30 | 90 | 8-12 |
| Prednisolone | 4 | 0.25 | 5 mg | 7,5-10 | 7,5 | 200 or> | 18-36 |
| Prednisone | 4 | 0.25 | 5 mg | 7,5-10 | 7,5 | 200 or> | 18-36 |
| Methylprednisolone | 5 | +/- | 4 mg | 7,5-10 | 7,5 | 200 or> | 18-36 |
| Triamcinolone | 5 | +/- | 4 mg | 7,5-10 | 7,5 | 200 or> | 18-36 |
| Paramethasone | 10 | +/- | 2 mg | 7,5-10 | 7,5 | 300 or> | 36-54 |
| Dexamethasone | 25 | +/- | 0,8mg | 1-1,5 | 2,5-5 | 300 or> | 36-54 |
| Betamethasone | 25-30 | +/- | 0,6mg | 1-1,5 | 1-1,5 | 300 or> | 36-54 |
| Aldosterone | 0.3 | 400 | - | - | - | 30 | |
| Fludrocortisone (flu-hydrocortisone) | 10 | 300 | - | - | - | 200 or> | 18-36 |

1. Relative milligram comparisons to cortisol, setting the glucocorticoid and mineralocorticoid properties of cortisol as 1. Sodium retention is insignificant with usually employed doses of methylprednisolone, triamcinolone, dexamethasone and betamethasone.
2. Intended as a guide only. The dose in an individual depends on total body surface area. The figures quoted are those which apply in general. (Adapted from Avery GS, Drug Treatment, 2nd edition, Churchill Livingsone, Eds. Edinburgh and London, p.530)

concentrations, necessary to inhibit PHA stimulated lymphocyte transformation by 50%, were measured. Potencies of hydrocortisone (set as 1.00), prednisolone, and dexamethasone were determined in 16 patients and were 1.00, 2.43, and 24.7, respectively.

Langhoff *et al*. used a similar method, comparing methylprednisolone, betamethasone, dexamethasone, hydrocortisone, prednisolone, and aldosterone [22]. Three classes of potencies were distinguished i.e. very potent: methylprednisolone and betamethasone; intermediate potent: dexamethasone and prednisolone, and low potent: hydrocortisone and aldosteron.

### *Competitive Protein- Binding Radioassays*

Angeli *et al*. used competitive protein-binding radioassays in various species and at different dilutions of transcortin-bound cortisol [23]. By comparing corticosterone, 11-deoxycortisol and prednisolone in the various species, only the binding ability of corticosterone was found to be particularly dependent upon the species.

### *Inhibition of Cytokine Production*

Snijdewint *et al*. investigated whether effects of GCs could be classified according to their distinct *in vitro* effects on T lymphocytes classes by studying $Th_1$- and $Th_2$-type cytokine production [24]. In the assay used, GC's concentrations in the range of $10^{-9}$ to $10^{-4}$M induced a class- and dose-dependent-inhibition of the production of both IFN-gamma and $IL_4$.

### *Eosinophilic Granulocyte Survival in Supernatant*

Mullol *et al*. discovered that supernatants from epithelial cell cultures enhance eosinophil survival *in vitro* [25]. This effect could be abrogated by foregoing incubation of peripheral blood eosinophils with GC's. Dexamethasone, methylprednisolone, deflazacort, and budesonide were tested in human epithelial cell conditioned media (HECM) obtained from healthy nasal mucosa- and polyp- cultures. Inhibitory potencies of the four steroids on the eosinophil survival index was compared using the concentration of each steroid that caused 50% survival inhibition ($IC_{50}$). Methylprednisolone was the least potent ($IC_{50}$ = 536nM), followed by deflazacort ($IC_{50}$= 264nM) and budesonide and dexamethasone (both $IC_{50}$=58nM), respectively. Notably, using the supernatant of nasal polyps revealed different steroid potencies i.e. methylprednisolone ($IC_{50}$=546nM), deflazacort ($IC_{50}$=390nM), dexamethasone ($IC_{50}$=76nM), and budesonide ($IC_{50}$=78nM).

## *In Vivo* Studies

### *Skin-Blanching Test*

The most well known *in vivo* test to determine the relative potencies of GC's (Table **1.2**) was the so called skin-blanching test by Mackenzie and Stoughton, which described vasoconstriction due to local application of GC's [26]. The test correlated with human CS receptor half-life and binding-affinities [27, 28]. Notably, a number of previously reported clinical trials have suggested that this skin-blanching test correlated fairly with various clinical efficacies (Table **1.2**). However these studies did not address concentration-effect relationships [29-33]. Moreover, a more recent study disputed the correlation between the skin-blanching test and the effects on airway and systemic hyper-responsiveness of inhaled corticosteroids [34].

### *Suppression of Cortisol Production*

Downie *et al*. performed a single-dose study to investigate the relative potency of predni-solone and betamethasone in suppressing adreno-cortical function [35]. Betamethasone produced a more profound suppression of plasma cortisol than a supposed equivalent anti-inflammatory dose of prednisolone.

### *Simultaneous Determinations of Systemic and Topical Effects*

In a study of McCubbin *et al*. local as well as systemic effects of inhaled beclomethasone-dipropionate (50µg/puff), triamcinolone-acetonide (100µg/puff), and flunisolide (250µg/puff) were investigated, looking at inhaled allergen suppression and urinary cortisol excretion [36]. All three inhaled corticosteroids (ICS) were described 'equipotent' in the doses administered towards local and systemic effects. Notably these findings were not sustained by data revealing concentration effect relationships and would have been different after correction for first pass effects of ICS in the liver [37]. Pharmacodynamic and pharmacokinetic (PK/PD) modelling studies of inhaled ICS are needed to overcome obvious confounders.

## *Ex Vivo* Studies

Distinct actions of CS on different effector organ systems have been previously reported. Monder *et al*. investigated 11 -hydroxysteroid-dehydrogenases (11 -HSD) production in Leydig cells in rat, dictating local GC's concentrations, which are related to testosterone secretion [38]. This group reported a half-maximal inhibition of testosterone production

**Table 1. 2. Relative Potencies of Various GC's According to the Skin-Blanching Test Compared to the Receptor Binding Expressed as Half Life and Affinity Hours**

| Drug | Topical potency skin-blanching (minutes) | CS-receptor binding half-life (hours) | CS-receptor binding affinity (hours) |
|---|---|---|---|
| Beclomethasone dipropionate (BDP) | 600 | 7.5 | 13.5 |
| Budesonide (BUD) | 980 | 5.1 | 9.4 |
| Flunisolide (FLU) | 330 | 3.5 | 1.8 |
| Fluticasone propionate (FP) | 1200 | 10.5 | 18 |
| Triamcinolone acetonide (TAA) | 330 | 3.9 | 3.6 |

Numbers are assigned in reference to dexamethasone, which has a value of "1" in the MacKenzie test.

for 1.5nM dexamethasone and for 0.4µM corticosterone. Notably, several compounds (i.e. glycyrrhetinic acid and mifepristone) exhibit distinct inhibitory actions on local cortisol concentrations via the 11 -HSD-system. It appeared, therefore, that the state of activation of local factors is of considerable importance to understand differential CS's potencies.

## Performed Clinical Studies

In a clinical study [39] the influence of hydrocortisone (HC) was described on two parameters, i.e. plasma tyrosine and lymphocyte count, demonstrating its genomic and nongenomic actions, respectively. After HC therapy, plasma tyrosine concentrations decrease as a result of induction of tyrosine amino transferase, a genomic (in-direct) effect. A decrease in lymphocyte count is considered to be a nongenomic (direct) effect. The objective of this study was to test the hypothesis that both genomic and nongenomic effects of HC can be described by essentially the same pharmacokinetic-pharmacodynamic (PK/PD) model. Seven healthy subjects were studied for two 24-hours periods during which they received either no drug (control day) or 300mg HC orally (test day). Plasma tyrosine concentration decreased by a mean maximum 15% on the control day and by 50% on the test day. Lymphocyte counts decreased by a mean maximum of 30% on the control day and by 75% on the test day. Tyrosine nadirs were observed on average two hours later than nadirs of lymphocyte counts. The mean HC concentration for 50% maximal drug effect ($EC_{50}$) was 378±186mg/L for plasma tyrosine levels. The mean estimated lymphocyte inhibition ($IC_{50}$) was 142±42mg/L. It was concluded that diurnal variations in tyrosine and lymphocytes can be described by fluctuations in levels of endogenous cortisol. The effects of endogenous cortisol and of exogenous HC on tyrosine and lymphocyte counts could be fitted to the same PK/PD model with identical parameters. This model may be used to describe concentration-dependent genomic and nongenomic actions of a variety of GCs.

In another clinical study [40] dexamethasone (DEXA) and prednisolone (PRED) were compared in assumed clinically equivalent doses with respect to the suppressive effect on the adrenal gland (cortisol). Induced effects towards cortisol, osteocalcin (OC), a parameter of bone metabolism and eosinophilic cationic protein (ECP), a parameter of inflammation, were simultaneously assessed. In four random sessions of 25 hours each, 8 healthy subjects received saline IV alone, Granulocyte-Colony Stimulating Factor (G-CSF) SC alone, or G-CSF in combination with either DEXA 2.0mg IV or PRED 12.5mg IV. A similar decrease in plasma cortisol concentration was observed up to 10 hours after DEXA and PRED administration; thereafter, a sustained suppression of plasma cortisol at 25 hours existed only after administration of DEXA. The AUC of OC was 24.4 and 2.3% lower than the AUC of OC without exogenous GC administration between 4 to 10 hours after DEXA and PRED administration. This suppression of OC also persisted at 25 hours, only after DEXA. The AUC of the G-CSF-stimulated ECP response decreased on average by 23.2% of baseline between 5 and 10 hours after administration of PRED, while no suppression was observed after DEXA ($p<0.02$). This suppression of ECP was no longer apparent at 25 hours. It was concluded that DEXA and PRED in formerly assumed clinically equivalent doses were equally suppressive towards cortisol within the first 10 hours after administration and exerted differential actions towards cortisol at 25 hours, OC and G-CSF-stimulated ECP.

The previous study was extended to PK/PD modelling studies to explain the clinical observations in healthy and asthmatic men [41]. At three random sessions of 25 hours each, 8 healthy and 6 asthmatic men received G-CSF SC alone, or G-CSF in combination with either DEXA 2.0mg IV or PRED 12.5mg IV. Induced effects towards cortisol, lymphocyte counts, OC and ECP were simultaneously studied. For each individual, all effect parameters could be fitted to a PK/PD model although for ECP adequate fitting was only possible in 7 healthy and 4 asthmatic men. Predicted endogenous cortisol reappeared in all healthy men within the observational period after PRED and in the asthmatic men after PRED and DEXA. Asthmatic men, who had stopped their regular ICS since a week, showed a predicted basal cortisol production of approximately 60% compared with healthy men. DEXA elicited a stronger maximal effect ($E_{max}$) towards all effect parameters except for ECP. $E_{max}$ of DEXA to suppress in suppressing ECP was higher in men with asthma compared with healthy individuals. The rank order of potencies ($EC_{50}$) was DEXA>PRED>cortisol for all effect parameters, regardless of differential clinical effects of each GC towards each parameter. PK/PD modelling revealed a higher $E_{max}$ of DEXA towards cortisol, lymphocyte counts and OC compared with that of PRED. Oppositely, $E_{max}$ of PRED towards ECP was higher than that of DEXA. Healthy and asthmatic men differed for each pharmacokinetic parameter studied. The AUC calculations for cortisol, lymphocyte counts and OC were in accordance with the estimated $EC_{50}$ values of PRED and DEXA. However, this was not the case for ECP, illustrating herein the paradoxical hindering effect of an agonist with a low activity (cortisol) in the presence of a relatively much stronger agonist (DEXA or PRED). The differences observed between healthy and asthmatic men cannot unequivocally be ascribed to prior ICS use, since asthma itself may play a role. Without further understanding, caution should be taken to determine potencies of GCs in asthmatic patients. It was concluded that DEXA and PRED in formerly assumed clinically equivalent doses were not equally potent towards different effect parameters although the ranking order of potencies were in agreement with the classical paradigm. Notably, pharmacokinetic outcomes were also different between healthy men and men with asthma who had stopped ICS for a week.

## CONCLUSIONS

Several glucocorticoids (GCs) exerted different actions towards various targets using the outcome parameters cortisol, lymphocyte counts, tyrosine, osteocalcine (OC), and eosinophilic cationic protein (ECP). The results of the performed clinical studies confirmed the hypothesis that the older bioassays, which led to the classical paradigm of

differential clinical efficacies of GCs, are inadequate for further studies. Moreover, studies on potencies of GCs should always direct towards PK/PD modelling of at least more than one biological target. Notably, more relevant clinical information was gained by studying a pro-inflammation marker (ECP) rather than classical outcome parameters of GC's action alone. For this purpose, a new model was developed i.e. ECP stimulation after Granulocyte Colony Stimulating Factor (G-CSF) SC surrogating a part of the asthmatic inflammatory response. This model proofed to be reproducible and useful. Hence a more realistic anti-inflammatory response of glucocorticoids in asthma could be compared with other *in vivo* responses.

## DIRECTIONS FOR FUTURE INVESTIGATIONS

Differential clinical effects and potencies of GCs were studied after systemic administration, orally or IV. Similar studies are needed in the field of inhaled corticosteroids (ICS) use in healthy individuals as well as in patients with pulmonary disease. To emphasize, these studies need to direct towards clinical outcome parameters as well as PK/PD modelling of various biological targets, thus describing a spectrum of potencies rather than potency.

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