Department of Pharmaceutical Sciences[1], College of Pharmacy, The University of Tennessee Health Science Center, Memphis, Tennessee; Department of Pharmaceutics[2], College of Pharmacy, University of Florida, Gainesville, Florida, USA

Transrepression and transactivation potencies of inhaled glucocorticoids

N. L. Dirks[1], S. Li[1], B. Huth[1], G. Hochhaus[2], C. R. Yates[1], B. Meibohm[1]

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Bernd Meibohm, PhD, FCP, Dpt. of Pharmaceutical Sciences, College of Pharmacy, The University of Tennessee Health Science Center, 874 Union Avenue, Suite 5p, Memphis, TN 38163, USA
bmeibohm@utmem.edu

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The anti-inflammatory activity of inhaled glucocorticoids is primarily mediated through transrepression of pro-inflammatory transcription factors such as AP-1 and NF-κB, while systemic side effects are largely attributed to transactivation via glucocorticoid response elements (GRE) in the promoter region of responsive genes. The objective of this study is to investigate whether inhaled corticosteroids exhibit differences in their transactivation and transrepression potencies. A549 human alveolar epithelial type II like cells, stably transfected with a reporter plasmid containing an AP-1, NF-κB or GRE induced secreted alkaline phosphatase reporter gene (SEAP), were exposed to a panel of concentrations of the six inhaled and three systemic glucocorticoids. Glucocorticoid-induced changes in SEAP expression were quantified by chemiluminescence. For eight glucocorticoids (budesonide, desisobutyryl-ciclesonide, dexamethasone, flunisolide, fluocortolone, fluticasone propionate, mometasone furoate, prednisolone) the EC_{50} for NF-κB mediated transrepression was significantly larger than that for both transactivation and transrepression via AP-1. For the remaining glucocorticoid (triamcinolone acetonide), it was greater than that for transactivation. It is concluded that, within the studied cell system, inhaled corticosteroids did not exhibit preferential transrepression, but had higher potencies for transactivation than for transrepression via NF-κB and had differential potencies for the two transrepression pathways.

1. Introduction

Inhaled glucocorticoids (GCs) are a cornerstone in the pharmacotherapeutic management of inflammatory lung diseases such as asthma (National Heart Blood and Lung Institute 2005). Topical administration by inhalation ensures high local anti-inflammatory activity in the lung, but limits undesired systemic activity (Hochhaus 2004). Nevertheless, the fraction of an inhaled GC dose deposited in the lung will be absorbed into the systemic circulation and is responsible for the known systemic side effects of inhaled GC therapy (Derendorf et al. 1998), including suppression of the hypothalamic-pituitary-adrenal axis, growth retardation and osteoporosis (Dahl 2006). Newer, more recently introduced inhaled GCs have been designed with properties that further support pulmonary targeting and minimize systemic activity, including increased pulmonary deposition efficiency and prolonged pulmonary residence time, low oral bioavailability to minimize uptake of drug swallowed during the inhalation process, and high systemic clearance for rapid inactivation once the GC has entered the systemic circulation (Derendorf et al. 1998; Hochhaus 2004).

After the key initial event of binding to the cytoplasmic glucocorticoid receptor (GR), GCs produce their effects on responsive cells by increasing or decreasing gene transcription through processes known as transactivation and transrepression, respectively (Barnes et al. 1998). Transactivation is mediated through the binding of an activated GR homodimer to glucocorticoid response elements (GRE) in the promoter region of responsive genes. Many of the systemic side effects associated with GC therapy (glaucoma, diabetes mellitus, hypertension, and osteoporosis) are likely mediated at least in part by transactivation (Schacke et al. 2002). Although GCs can induce gene transcription of several anti-inflammatory mediators such as lipocortin-1, interleukin-1 receptor antagonist, and interleukin-10, it is unlikely that the wide-spread anti-inflammatory action of GCs is due to the limited number of anti-inflammatory genes controlled by transactivation (Barnes and Adcock 2003; Pelaia et al. 2003). In addition, negative GREs have not been described in the promoter regions of inflammatory genes suppressed by GCs (Barnes and Adcock 2003). Thus, the anti-inflammatory effect of GCs is assumed to be largely due to transrepression of inflammatory genes through GR-mediated inhibition of pro-inflammatory transcription factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) (De Bosscher et al. 2003; Hayashi et al. 2004). Many of the genes encoding inflammatory mediators such as cytokines, chemokines, and adhesion molecules are controlled by NF-κB and AP-1 (Barnes and Karin 1997; Barnes and Adcock 1998).

A preferential transrepression compared to transactivation may potentially reduce the risk of systemic side effects

and could thus be a major advantage in inhaled GC therapy. Transactivation and transrepression potencies of GCs have previously been studied using gene reporter assays and electrophoretic mobility shift assays (Smith and Kreutner 1998; Adcock et al. 1999; Jaffuel et al. 2000; Jaffuel et al. 2001; Austin et al. 2002; Roumestan et al. 2003), but investigations were limited to only a small number of GCs per study and the applied methodologies did not allow for an absolute comparison between transactivation and transrepression potencies.

To address the question whether select inhaled corticosteroids exhibit preferential transrepression, the present study determined the transactivation and transrepression potencies of six topical GCs currently approved in North America and/or Europe for inhalation pharmacotherapy using human lung epithelial (A549) cells stably transfected with AP-1, NF-κB, or GRE dependent reporter plasmids: budesonide, ciclesonide, flunisolide, fluticasone propionate, mometasone furoate, and triamcinolone acetonide. Three systemically administered GCs were investigated as reference: dexamethasone, fluocortolone, and prednisolone.

2. Investigations and results

The transactivation and transrepression potencies could be determined for all studied GCs in all three cell systems. By using several concentrations over a wide range, the concentration-effect profile for each GC was well characterized, as exemplified for fluticasone propionate in each cell line (Fig. 1). As expected, repression of AP-1 and NF-κB activity in the cell lines containing the pAP-1-SEAP and pNF-κB-SEAP-NPT reporter plasmids resulted in a decrease in SEAP expression and luminescence signal from baseline (Fig. 1A and B). In the cell line containing the pGRE-SEAP reporter plasmid, an increase in SEAP expression and luminescence signal was seen due to transactivation (Fig. 1C). The average maximal inhibition of AP-1 and NF-κB activity and activation of GRE from baseline was 43%, 40%, and 182% respectively. There were no statistically significant differences in maximum effect between GCs in individual cell lines based on a one-way ANOVA analysis (results not shown).

The transactivation and transrepression potencies for the investigated GCs quantified as EC_{50}, as well as their literature reported relative receptor affinity (RRA; relative to dexamethasone [RRA = 100]) are listed in the Table (Rohdewald et al. 1986; Würthwein et al. 1992; Rohatagi et al. 2003; Valotis et al. 2004). In analogy to relative receptor affinity, Table 1 also includes the relative potency for transactivation and transrepression calculated based on the EC_{50} values observed in this study. In general, potency rankings for transactivation and transrepression matched the RRA ranking, except for several cases where GCs with neighboring rank switched positions.

In each cell line an inverse relationship between RRA and EC_{50} was observed (Fig. 2), indicating a positive correlation between RRA and potency for both transrepression and transactivation. This relationship appeared linear for GCs with lower RRAs as indicated by the insert graphs in Fig. 2. These insert graphs represent a range of RRA from 16 (prednisolone) to 935 (budesonide). However, with the inclusion of all GCs studied, the relationship between RRA and $1/EC_{50}$ appears curvilinear. In several cases, the GCs with RRA greater than 1000 (desisobutyryl-ciclesonide, fluticasone propionate, mometasone furoate) exhibited EC_{50} values lower than predicted by RRA alone. This was especially apparent for mometasone furoate in each of the cell lines, but was not observed with desisobutyryl-ciclesonide and fluticasone propionate in the NF-κB cell line.

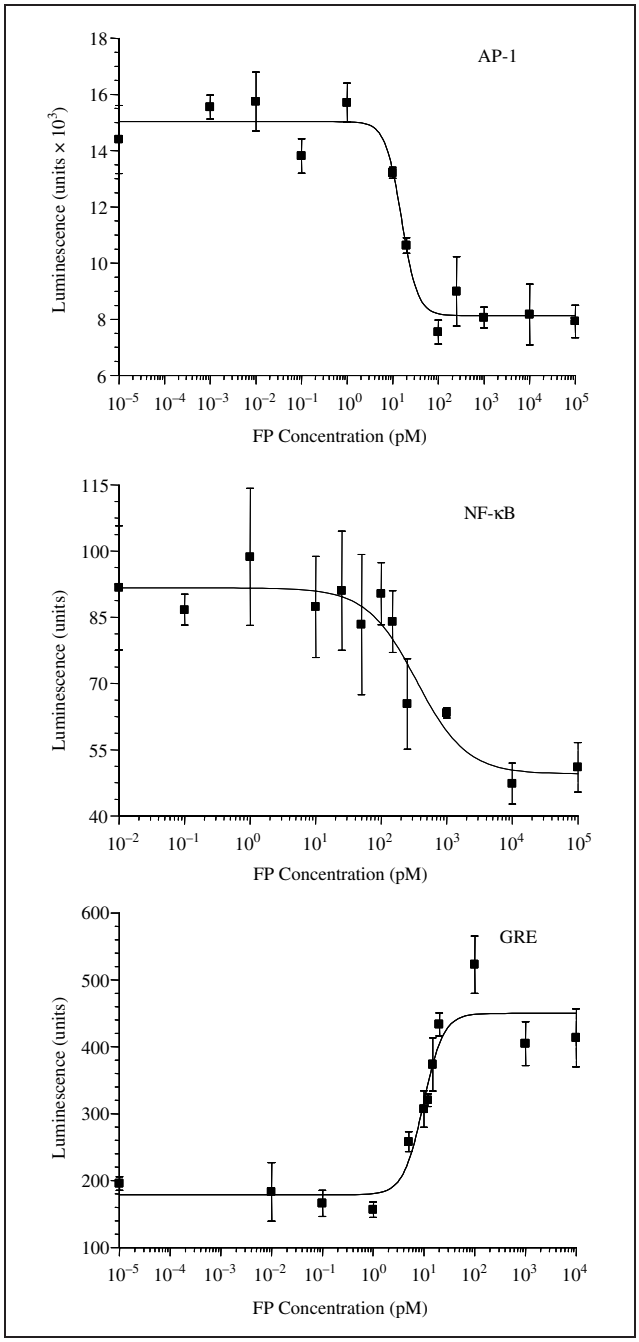

Fig. 1: Concentration-effect profiles of fluticasone propionate (FP) for AP-1, NF-κB, and GRE. Each point represents the mean ± SD luminescence signal (arbitrary units) of three measurements. For graphical purposes, results shown for 10^{-5} pM are those for the vehicle control. The solid line represents the curve described by the sigmoid E_{max} model (Eq. (1)).

The ability of individual GCs to differentiate between transactivation and transrepression was investigated by comparison of their respective potencies in the three transfected cell lines and is presented in Fig. 3. For all GCs, the EC_{50} for transrepression via NF-κB was significantly greater than that for transactivation. Thus, all investigated GCs have a higher potency for transactivation than NF-κB-mediated transrepression in the transfected A549 cell lines. In only two cases, desisobutyryl-ciclesonide and budesonide, the transactivation potency was significantly

Table: Glucocorticoid relative receptor affinities (RRA) literature reported values* as well as estimated EC_{50} values (mean ± SD) and relative potencies for transrepression (AP-1, NF-κB) and transactivation (GRE)

Glucocorticoid	RRA	GRE		NF-κB		AP-1		Transactivation/-repression potencies	
		EC_{50} (pM)	Relative potency	EC_{50} (pM)	Relative potency	EC_{50} (pM)	Relative potency	NF-κB vs. GRE	AP-1 vs. GRE
Prednisolone	16	4320 (198)	21.0	16969 (596)	12.4	4705 (484)	7.77	3.9	1.1
Fluocortolone	82	744 (279)	**122**	7847 (2063)	26.8	839 (324)	43.6	11	1.1
Dexamethasone	100	907 (131)	**100**	2106 (479)	100	366 (34.6)	**100**	2.3	0.4
Flunisolide	180	289 (33.1)	**313**	1563 (630)	135	309 (170)	**118**	5.4	1.1
Triamcinolone acetonide	233	300 (39.3)	**302**	830 (392)	254	524 (34.5)	**69.7**	2.8	1.7
Budesonide	935	90.3 (0.4)	1005	289 (5.2)	**729**	130 (9.4)	280	3.2	1.4
Desisobutyryl-ciclesonide	1210	10.3 (0.7)	**8785**	729 (215)	**289**	67.6 (4.0)	540	71	6.5
Fluticasone propionate	1800	10.6 (1.2)	**8572**	327 (28)	**645**	29.0 (20.4)	1262	31	2.7
Mometasone furoate	2900	9.29 (3.01)	9763	37.2 (2.1)	5662	8.25 (3.45)	4428	4.0	0.89

* (Rohdewald et al. 1986; Würthwein et al. 1992; Rohatagi et al. 2003; Valotis et al. 2004)
Relative potency for any glucocorticoid (GC) was calculated as $(EC_{50,Dexamethasone}/EC_{50,GC}) \times 100$. Relative transactivation-to-transrepression potencies were calculated as $(EC_{50,NF\text{-}\kappa B \text{ or } AP\text{-}1}/EC_{50,GRE})$. Bold numbers designate relative potencies that do not parallel the glucocorticoid ranking based on RRA

higher than AP-1 mediated transrepression. In addition, all investigated GCs except for triamcinolone acetonide had a significantly higher potency for AP-mediated transrepression than for NF-κB mediated transrepression. None of the investigated GCs, except dexamethasone, exhibited a higher potency for either AP-1 or NF-κB mediated transrepression than for transactivation. This observation is also

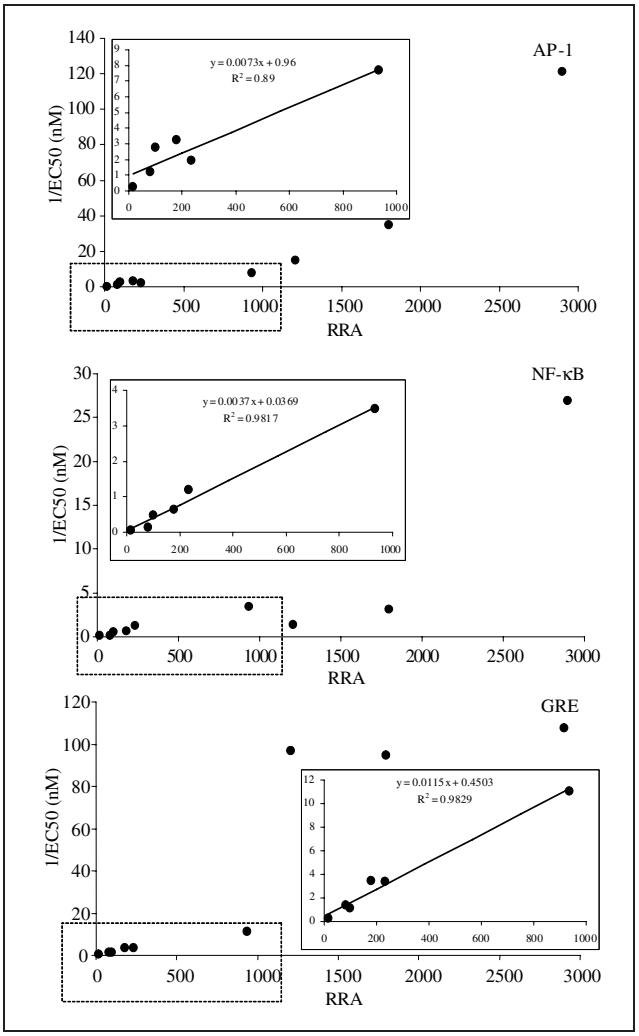

Fig. 2: Plot of glucocorticoid relative receptor affinity (RRA) versus inverse EC_{50} values (nM) from the Table for AP-1, NF-κB, and GRE. Data within the dashed box are shown as an insert graph.

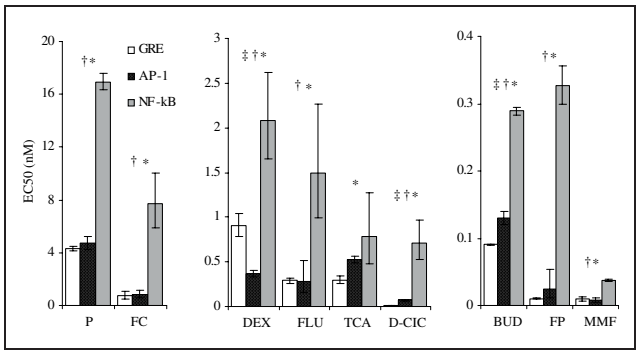

Fig. 3: Comparison of transrepression and transactivation potencies (mean ± geometric SD) quantified as EC_{50} across the three cell lines for individual glucocorticoids. The graph is split into three panels with different EC_{50} ranges for presentation purposes ($^{*}p < 0.05$ NF-κB vs. GRE, $^{‡}p < 0.05$ AP-1 vs. GRE, $^{†}p < 0.05$ NF-κB vs. AP-1). P prednisolone; FC fluocortolone; DEX dexamethasone; FLU flunisolide; TCA triamcinolone acetonide; D-CIC desisobutyryl-ciclesonide; BUD budesonide; FP fluticasone propionate; MMF mometasone furoate

reflected in the relative transactivation-to-transrepression potencies listed in the Table.

3. Discussion

GCs are known to modulate gene expression through transactivation and transrepression. While the majority of the anti-inflammatory effects of GCs is believed to come from inhibiting the actions of pro-inflammatory transcription factors such as AP-1 and NF-κB, systemic side effects of inhaled corticosteroid therapy are largely attributed to transactivation (Dahl 2006). Thus, a preferential transrepression compared to transactivation may potentially reduce the risk of systemic side effects in inhaled GC therapy and increase their risk-benefit ratio, although there is so far no evidence that an optimized transactivation-to-transrepression ratio will have beneficial clinical effects (Hochhaus 2004).

In the present study, we determined the transcriptional potencies of six inhaled GCs and three systemically administered GCs by using A549 cells stably transfected with reporter plasmids containing an AP-1, NF-κB, or GRE dependent promoter linked to the SEAP gene. Our results indicate that none of the studied inhaled GCs has a higher transrepression than transactivation potency. In fact, there is a general trend for larger EC_{50} values for the NF-κB

cell line versus the other two cell lines, translating into a lower potency for NF-κB mediated transrepression compared to AP-1 mediated transrepression or transactivation. For five of the six inhaled GCs as well as the three systemic GCs, the EC_{50} for inhibition of NF-κB activity was significantly greater than that for inhibition of AP-1 activity and transactivation ($p < 0.05$). In the remaining GC, triamcinolone acetonide, a statistically significant difference in EC_{50} was observed between NF-κB and GRE. This suggests that, after GR activation in A549 cells, there is preferential targeting of AP-1 and GRE as compared to NF-κB.

The selective transrepression of AP-1 activity versus NF-κB activity observed in this study is an interesting finding. Transrepression is most likely mediated by GR monomers, whereas dimerization of the GR is required for transactivation (Belvisi et al. 2001; Pelaia et al. 2003). The ability of the GR monomer to distinguish between AP-1 and NF-κB suggests that different mechanisms and/or molecular regions are involved in the repression of these transcription factors. This hypothesis is further supported by a recent finding of a GR mutant that has an impaired ability to repress stimulated NF-κB activity, but its AP-1 repression activity remained intact (Bladh et al. 2005).

The results of our study on relative transcriptional potencies differ significantly from several previous studies that have investigated the transcriptional potencies of inhaled GCs using gene reporter assays (Smith and Kreutner 1998; Adcock et al. 1999; Jaffuel et al. 2000; Austin et al. 2002; Roumestan et al. 2003) and reported higher GC concentrations needed for transactivation versus transrepression (Adcock et al. 1999; Jaffuel et al. 2000; Roumestan et al. 2003). The reason for these different observations is questionable, but may be related to the use of different cell types, reporter constructs, and cell stimuli. Only a small number of GCs was investigated in each of these studies and the applied methodologies did not allow for an absolute comparison between transactivation and transrepression potencies. In one study, for example, stably transfected HeLa S3 cells were used to study transactivation whereas transiently transfected A549 cells were used to measure repression of AP-1 and NF-κB activity (Jaffuel et al. 2000). In addition, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) was used to stimulate AP-1 activity, while NF-κB activity was enhanced by overexpressing the p65 subunit. In another study, transactivation was measured by electrophoretic mobility shift assays, while transrepression of AP-1 and NF-κB was assessed by reporter gene assays (Adcock et al. 1999). The use of different cell lines, assays, and enhancers of NF-κB and AP-1 activity, as well as the transfection status (transient vs. stable) could all have an impact on observed differences in transcriptional potencies between AP-1, NF-κB, and GRE.

In the current study, only one cell line was used, cells were stably transfected with the respective reporter plasmids, and experimental conditions were kept similar for each cell system in order to allow for comparisons. Using SEAP as the reporter gene offered several advantages, including the ability to measure activity by sampling media rather than using cell lysates. Furthermore, the SEAP-based chemiluminescence assay is reportedly ten times more sensitive then luciferase assays (Yang et al. 1997). The cell line used for transfection was selected based on the focus of this study on inhaled GCs used for the pharmacotherapy of asthma. Distal airways of the lung, including the alveoli, likely play a role in the pathogenesis of asthma and serve as a source of inflammatory mediators (Dobbs 1994; Kraft 1999; Martin 2002), and thus, the A549 human alveolar type II epithelium-like cell line was considered appropriate for this study. While the expression levels of SEAP varied between the three cell systems, the observed EC_{50} values as measures of potency are not expected to be influenced by these differences. However, the differences in expression will affect the observed E_{max}, and thus comparisons between GCs with regard to E_{max} were only made within an individual cell system.

It is well accepted that the potency for a given effect varies between GCs and that potency correlates with RRA. *In vitro*, this has been observed for transactivation and transrepression (AP-1, NF-κB) (Smith and Kreutner 1998; Jaffuel et al. 2000), as well as other GC mediated events such as induction of CD163 and inhibition of basophil histamine release, IL-4 and IL-5 secretion, eosinophil viability, expression of vascular cell adhesion molecule-1 (VCAM-1), and lymphocyte proliferation (Umland et al. 1997; Högger et al. 1998; Atsuta et al. 1999; Stellato et al. 1999; Mager et al. 2003; Valotis et al. 2004). *In vivo*, good inverse correlations between RRA and EC_{50} as potency measure have been described for cortisol suppression and inhibition of lymphocyte trafficking (Derendorf et al. 1997; Rohatagi et al. 2003). The results of the present study coincide with these reports as an inverse correlation between RRA and EC_{50} was observed in each of the three cell systems (Fig. 2). Although this relationship appeared to be linear up to a RRA of ~1000, mometasone furoate, for example, exhibited a much lower EC_{50} than expected based on RRA in all cell lines. This observation of a curvilinear relationship observed between RRA and $1/EC_{50}$ is not unique to the present study. Although not explicitly reported, the results from other studies investigating transcriptional potencies, inhibition of VCAM-1 expression, inhibition of IL-4 and IL-5 secretion, and induction of CD163, also exhibit a curvilinear relationship between RRA and $1/EC_{50}$ when graphed (Umland et al. 1997; Smith and Kreutner 1998; Atsuta et al. 1999; Jaffuel et al. 2000; Valotis et al. 2004). A potential reason for this curvilinear relationship may be that in addition to RRA, the activity of GCs may depend on their ability to alter the GR conformation in a ligand dependent manner. These conformational changes in the GR could have an impact on its interaction with DNA, co-activators and -repressors, and other transcriptional factors. Another possible explanation for the curvilinear relationship is the experimental variability associated with estimating RRA, which may have resulted in an underestimation of the RRA for GCs with high potency such as mometasone furoate.

Several immunoregulatory genes including VCAM-1, TNF-α (tumor necrosis factor α), RANTES (regulated upon activation, normal T-cell expressed and secreted), and GM-CSF (granulocyte-macrophage colony stimulating factor), contain NF-κB and/or AP-1 sites in their promoters or regulatory regions (De Bosscher et al. 2003). Several studies have investigated the potency of various GCs to inhibit the expression of the aforementioned immunoregulatory genes (Adcock et al. 1999; Atsuta et al. 1999; Stellato et al. 1999; Jaffuel et al. 2000; Salter et al. 2007). While the EC_{50} values observed in these studies differ from those for transrepression of AP-1 and NF-κB activity in the current study, in general, the rank potencies for transrepression coincided with those for inhibition of VCAM-1, TNF-α, RANTES, and GM-CSF expression. The potency for GCs to transactivate the expression of CD163 and the β_2-adrenergic receptor have also been in-

vestigated (Högger et al. 1998; Adcock et al. 1999; Valotis et al. 2004), and again while the EC_{50} values differed from those observed in this study for transactivation, the rank potencies were in agreement with those for increasing expression of CD163 and the β_2-receptor.

Whether or not preferential targeting of AP-1 and GRE observed in A549 cells can be extrapolated to other cell types is questionable. Potency differences between cell types can exist due to characteristics such as histone deacetylase activity, modulation of GR activity by corepressors and coactivators, and altered GC affinity due to the phosphorylation status of the GR (Czock et al. 2005). Transactivation in the transfected A549 cells of this study occurred at concentrations similar to transrepression of AP-1 for most studied GCs. Transactivation likely plays a role in many of the systemic side effects seen with GC therapy. However, the results presented here were observed in human alveolar type II epithelial cells which are during clinical pharmacotherapy with inhaled GCs not involved in producing systemic side effects and in which transactivation may even have a beneficial role. Indeed, there are a number of GC inducible genes with anti-inflammatory effects including MAP kinase phosphatase 1 (MKP-1), Clara cell secretory protein, glucocorticoid-inducible leucine zipper (GILZ), IL-1 receptor antagonist, lipocortin-1, and inhibitor of NF-κB (IκBα) (Hayashi et al. 2004; Newton and Holden 2007). As new GC ligands are being developed with preferential transrepression versus transactivation properties in an attempt to improve their risk-benefit ratio, based on the results of this study, this improvement in risk-benefit ratio should be carefully balanced against the potential loss of anti-inflammatory effects. As suggested by others, "desirable" and "undesirable" transactivation and transrepression events need to be defined, and rather than develop GCs that simply differentiate between transactivation and transrepression, they should be functionally screened to identify "differential" compounds with favorable functional profiles (Newton and Holden 2007).

In summary, the current investigation provides no indication that the currently available inhaled corticosteroids exhibit preferential transrepression compared to transactivation. In fact transactivation potency was of similar or higher magnitude as AP-1 mediated transrepression potency for all investigated inhaled GCs, and was significantly higher than the NF-κB mediated transrepression potency.

4. Experimental

4.1. Glucocorticoids

Budesonide (Sicor, Milan, Italy), dexamethasone (Merck, Darmstadt, Germany), flunisolide (Roche Diagnostics, Mannheim, Germany), fluocortolone (Schering, Berlin, Germany), fluticasone propionate (GlaxoSmithKline, Research Triangle Park, NC), prednisolone (SanofiAventis, Frankfurt, Germany), and triamcinolone acetonide (SanofiAventis, Frankfurt, Germany) were obtained from the indicated sources. Ciclesonide was isolated from an Alvesco® metered dose inhaler (Altana Pharma, Konstanz, Germany). Mometasone furoate was purchased from United States Pharmacopeia (Rockville, MD). All GCs were initially dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO), and subsequent serial dilutions with DMSO were stored at −20 °C.

4.2. Cell lines

A549 cells (human alveolar type II epithelium-like cell line; American Type Culture Collection, Manassas, VA) stably transfected with AP-1, NF-κB, or GRE dependent reporter plasmids were provided by the laboratory of Dr. Charles R. Yates (University of Tennessee, Memphis, TN). The NF-κB cell line was produced by transfecting A549 cells with the pNF-κB-SEAP-NPT plasmid donated by Dr. Yeong Shik Kim (Seoul National University, Seoul, Korea) (Moon et al. 2001). This plasmid contains the neomycin phosphotransferase (NPT) gene for geneticin resistance, in addition to four tandem copies of the NF-κB consensus sequence fused upstream of a TATA-like region (P_{TAL}) from the Herpes simplex virus thymidine kinase (HSV-TK) promoter. The AP-1 and GRE cell lines were created by co-transfecting A549 cells with the reporter plasmids pAP-1-SEAP or pGRE-SEAP and the pPUR plasmid conferring puromycin resistance (BD Biosciences Clontech, Palo Alto, CA). The pAP-1-SEAP and pGRE-SEAP plasmids contained either four tandem copies of the AP-1 consensus sequence or three tandem copies of the GRE sequence, respectively, fused upstream of a TATA-like region (P_{TAL}) from the HSV-TK promoter. Each reporter plasmid consisted of an AP-1, NF-κB, or GRE dependent promoter linked to the secreted alkaline phosphatase gene (SEAP). The three cell lines were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% heat inactivated fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and either 10 μg/mL geneticin or 1 μg/mL puromycin.

4.3. SEAP Reporter gene assay

A SEAP-based reporter assay was used to quantify transactivation and transrepression activities of the investigated GCs. Expression of the SEAP gene in the transfected A549 cells was modulated by GC addition through interaction of the activated GR with AP-1, NF-κB or the GRE. As SEAP is secreted into the culture medium in proportion to its mRNA levels in the transfected cells, SEAP activity in the medium quantified by chemiluminescence was used to quantify the GC effect on the respective cell system. All measurements used for this analysis were within the linear range of the SEAP assay.

Transfected cells were seeded at 25,000 cells/well into 24-well cluster plates, and incubated at 37 °C. After 24 h, the media was removed and each well was washed with Dulbecco's Modified Eagle Medium. Fresh media was added, supplemented as described above, but rather 1% fetal bovine serum was used. Fetal bovine serum serves to stimulate AP-1 and NF-κB activity. The concentration of 1% was found to maximally stimulate AP-1 activity (data not shown), and subsequently was used for the NF-κB and GRE cell lines to keep experimental conditions the same. The cells were treated with vehicle or varying GC concentrations of 10^{-5} M to 10^{-14} M depending on the GC. The final concentration of DMSO per well was 1%. For ciclesonide, porcine liver esterase (0.5 U/well) was used to convert it to its active metabolite desisobutyryl-ciclesonide. After treatment, the cells were incubated for an additional 24 h at 37 °C. Media was sampled (50 μL), and SEAP activity was detected in a 96-well black plate by chemiluminescence using the Great Escape SEAP Chemiluminescence Detection Kit (BD Biosciences Clontech, Palo Alto, CA) on a luminometer (DTX 880, Beckman Coulter, Fullerton, CA).

4.4. Pharmacodynamic and statistical analysis

Each experiment was repeated twice, with four additional concentrations added in the second experiment to more accurately estimate the GC concentration that produces the half-maximal effect (EC_{50}). This resulted in a concentration-effect profile characterized by twelve concentrations (including the vehicle control), each done in triplicate. The EC_{50} as a measure of potency for transrepression or transactivation was estimated from the concentration-effect data for each individual experiment by nonlinear regression analysis (Scientist v.2.0; MicroMath, St. Louis, MO) using a sigmoid E_{max} model:

$$E = E_0 \pm E_{max} \times C^n / (EC_{50}^n + C^n)$$

where E is the incremental change in chemiluminescence signal from baseline as a measure of GC effect, E_0 is the chemiluminescence signal in the absence of GC (vehicle control), E_{max} is the maximum incremental chemiluminescence change under GC exposure, C is the nominal GC concentration in the medium, and n is the Hill coefficient.

GCs exhibit moderate to high levels of plasma protein binding, with bound fractions of approximately 70–90% (Derendorf et al. 1998; Czock et al. 2005). As only unbound GC can interact with the GR, the reported EC_{50} values were converted to unbound concentrations. Since a low amount of FBS (1%) was used in this experiment, however, the EC_{50} values for total and unbound concentrations were similar and only one set of EC_{50} values is reported.

Differences in the EC_{50} values for transrepression and transactivation of individual GCs were assessed after log-transformation using ANOVA (SAS v.9.1; SAS Institute, Cary, NC). Statistical significance was considered as $p < 0.05$.

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References

Adcock IM, Nasuhara Y, Stevens DA, Barnes PJ (1999) Ligand-induced differentiation of glucocorticoid receptor (GR) trans-repression and transactivation: preferential targetting of NF-kappaB and lack of I-kappaB involvement. Br J Pharmacol 127: 1003–1011.

Atsuta J, Plitt J, Bochner BS, Schleimer RP (1999) Inhibition of VCAM-1 expression in human bronchial epithelial cells by glucocorticoids. Am J Respir Cell Mol Biol 20: 643–650.

Austin RJ, Maschera B, Walker A, Fairbairn L, Meldrum E, Farrow SN, Uings IJ (2002) Mometasone furoate is a less specific glucocorticoid than fluticasone propionate. Eur Respir J 20: 1386–1392.

Barnes PJ, Adcock IM (1998) Transcription factors and asthma. Eur Respir J 12: 221–234.

Barnes PJ, Adcock IM (2003) How do corticosteroids work in asthma? Ann Intern Med 139: 359–370.

Barnes PJ, Karin M (1997) Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 336: 1066–1071.

Barnes PJ, Pedersen S, Busse WW (1998) Efficacy and safety of inhaled corticosteroids. New developments. Am J Respir Crit Care Med 157: S1–53.

Belvisi MG, Brown TJ, Wicks S, Foster ML (2001) New Glucocorticosteroids with an improved therapeutic ratio? Pulm Pharmacol Ther 14: 221–227.

Bladh LG, Liden J, Dahlman-Wright K, Reimers M, Nilsson S, Okret S (2005) Identification of endogenous glucocorticoid repressed genes differentially regulated by a glucocorticoid receptor mutant able to separate between nuclear factor-κB and activator protein-1 repression. Mol Pharmacol 67: 815–826.

Czock D, Keller F, Rasche FM, Haussler U (2005) Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. Clin Pharmacokinet 44: 61–98.

Dahl R (2006) Systemic side effects of inhaled corticosteroids in patients with asthma. Respir Med 100: 1307–1317.

De Bosscher K, Vanden Berghe W, Haegeman G (2003) The interplay between the glucocorticoid receptor and nuclear factor-κB or activator protein-1: molecular mechanisms for gene repression. Endocr Rev 24: 488–522.

Derendorf H, Hochhaus G, Meibohm B, Mollmann H, Barth J (1998) Pharmacokinetics and pharmacodynamics of inhaled corticosteroids. J Allergy Clin Immunol 101: S440–446.

Derendorf H, Mollmann H, Hochhaus G, Meibohm B, Barth J (1997) Clinical PK/PD modelling as a tool in drug development of corticosteroids. Int J Clin Pharmacol Ther 35: 481–488.

Dobbs LG (1994) Alveoli and airways: possible interactions in the pathogenesis of asthma. Am J Respir Crit Care Med 150: S31–32.

Hayashi R, Wada H, Ito K, Adcock IM (2004) Effects of glucocorticoids on gene transcription. Eur J Pharmacol 500: 51–62.

Hochhaus G (2004) New developments in corticosteroids. Proc Am Thorac Soc 1: 269–274.

Högger P, Erpenstein U, Rohdewald P, Sorg C (1998) Biochemical characterization of a glucocorticoid-induced membrane protein (RM3/1) in human monocytes and its application as model system for ranking glucocorticoid potency. Pharm Res 15: 296–302.

Jaffuel D, Demoly P, Gougat C, Balaguer P, Mautino G, Godard P, Bousquet J, Mathieu M (2000) Transcriptional potencies of inhaled glucocorticoids. Am J Respir Crit Care Med 162: 57–63.

Jaffuel D, Roumestan C, Balaguer P, Henriquet C, Gougat C, Bousquet J, Demoly P, Mathieu M (2001) Correlation between different gene expression assays designed to measure trans-activation potencies of systemic glucocorticoids. Steroids 66: 597–604.

Kraft M (1999) The distal airways: are they important in asthma? Eur Respir J 14: 1403–1417.

Mager DE, Moledina N, Jusko WJ (2003) Relative immunosuppressive potency of therapeutic corticosteroids measured by whole blood lymphocyte proliferation. J Pharm Sci 92: 1521–1525.

Martin RJ (2002) Therapeutic significance of distal airway inflammation in asthma. J Allergy Clin Immunol 109: S447–460.

Moon KY, Hahn BS, Lee J, Kim YS (2001) A cell-based assay system for monitoring NF-κB activity in human HaCat transfectant cells. Anal Biochem 292: 17–21.

National Heart Blood and Lung Institute (2005) Global Initiative for Asthma – Global Strategy for Asthma Management and Prevention. National Institutes of Health, Bethesda, MD.

Newton R, Holden NS (2007) Separating transrepression and transactivation: a distressing divorce for the glucocorticoid receptor? Mol Pharmacol 72: 799–809.

Pelaia G, Vatrella A, Cuda G, Maselli R, Marsico SA (2003) Molecular mechanisms of corticosteroid actions in chronic inflammatory airway diseases. Life Sci 72: 1549–1561.

Rohatagi S, Arya V, Zech K, Nave R, Hochhaus G, Jensen BK, Barrett JS (2003) Population pharmacokinetics and pharmacodynamics of ciclesonide. J Clin Pharmacol 43: 365–378.

Rohdewald P, Mollman HW, Hochhaus G (1986) Affinities of glucocorticoids for glucocorticoid receptors in the human lung. Agents Actions 17: 290–291.

Roumestan C, Henriquet C, Bousquet J, Mathieu M (2003) Fluticasone propionate and mometasone furoate have equivalent transcriptional potencies. Clin Exp Allergy 33: 895–901.

Salter M, Biggadike K, Matthews JL, West MR, Haase MV, Farrow SN, Uings IJ, Gray DW (2007) Pharmacological properties of the enhanced-affinity glucocorticoid fluticasone furoate in vitro and in an in vivo model of respiratory inflammatory disease. Am J Physiol Lung Cell Mol Physiol 293: L660–667.

Schacke H, Docke WD, Asadullah K (2002) Mechanisms involved in the side effects of glucocorticoids. Pharmacol Ther 96: 23–43.

Smith CL, Kreutner W (1998) In vitro glucocorticoid receptor binding and transcriptional activation by topically active glucocorticoids. Arzneimittelforschung 48: 956–960.

Stellato C, Atsuta J, Bickel CA, Schleimer RP (1999) An in vitro comparison of commonly used topical glucocorticoid preparations. J Allergy Clin Immunol 104: 623–629.

Umland SP, Nahrebne DK, Razac S, Beavis A, Pennline KJ, Egan RW, Billah MM (1997) The inhibitory effects of topically active glucocorticoids on IL-4, IL-5, and interferon-gamma production by cultured primary CD4+ T cells. J Allergy Clin Immunol 100: 511–519.

Valotis A, Neukam K, Elert O, Hogger P (2004) Human receptor kinetics, tissue binding affinity, and stability of mometasone furoate. J Pharm Sci 93: 1337–1350.

Würthwein G, Rehder S, Rohdewald P (1992) Lipophilicity and receptor affinity of glucocorticoids. Pharm Ztg Wiss 5/137: 161–167.

Yang TT, Sinai P, Kitts PA, Kain SR (1997) Quantification of gene expression with a secreted alkaline phosphatase reporter system. Biotechniques 23: 1110–1114.