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# Fluticasone propionate and mometasone furoate have equivalent transcriptional potencies

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## Summary

*Background* Glucocorticoids exert their anti-inflammatory effects mainly through transrepression of the transcription factors activator protein-1 (AP-1) and nuclear factor-kappa B (NF-κB). Certain adverse effects of glucocorticoids are mediated through gene transactivation. Fluticasone propionate (FP) and mometasone furoate (MF) are the most recently developed topical glucocorticoids for the treatment of airway disorders. Their relative capacities to repress AP-1 and NF-κB activities are not known and comparison of their transactivation potencies has given unclear results.

*Objective* To determine the relative transactivation and transrepression potencies of FP and MF.

*Methods* Transactivation assays were performed in HeLa cells carrying a glucocorticoid-inducible luciferase gene. To measure transrepressive potencies of FP and MF, A549 lung epithelial cells were transiently transfected with an AP-1- or NF-κB-dependent luciferase gene. Using an immunoassay, we also evaluated the ability of MF and FP to inhibit the production of Regulated upon Activation, Normal T-cell Expressed and Secreted (RANTES), a pro-inflammatory cytokine, whose gene is controlled by AP-1 and NF-κB. Areas under the dose–response curve were calculated to determine relative potencies.

*Results* FP and MF are equipotent for transactivation. Both molecules show globally the same potency to inhibit AP-1 and NF-κB activities and RANTES production. MF and FP have very significant transcriptional effects at $2 \times 10^{-10}$ M, which is the peak concentration reached in the plasma after inhalation of high dosages. Indeed, they produce a 17-fold induction of luciferase in the transactivation assay, and inhibit AP-1 activity, NF-κB activity and RANTES release by approximately 40%.

*Conclusion* FP and MF have the same ability to trigger gene activation and also the same potency to inhibit AP-1 and NF-κB activities. Their strong transcriptional effects at $2 \times 10^{-10}$ M suggest that these compounds act not only topically but also systemically, with the risk of provoking concomitant adverse effects at high dosages.

**Keywords** AP-1, glucocorticoids, NF-κB, RANTES, reporter gene, topical, transactivation, transrepression.

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## Introduction

Glucocorticoids (GCs) are commonly used to treat chronic inflammatory diseases. After binding to the GC receptor α (GRα), these hormones can increase or inhibit gene expression through processes called transactivation and transrepression, respectively.

Transactivation occurs after binding of hormone-activated GRα to a gene regulatory sequence called GC response element (GRE). GCs transactivate the $\beta_2$-adrenergic receptor gene and may, consequently, facilitate bronchodilatory action of $\beta_2$-agonists [1, 2]. However, most genes transactivated by GCs are likely involved in mediating certain side effects including hypertension, oedema, hypokalaemia, glaucoma and diabetes [3–8].

The transcription factors AP-1 and NF-κB induce the expression of an array of genes encoding inflammatory mediators, and are themselves activated by inflammatory stimuli [9, 10]. In the past decade, it was demonstrated that the anti-inflammatory effects of GCs are predominantly mediated through transrepression of AP-1 and NF-κB activities [9, 11].

We have recently shown that among the five inhaled GCs available for the treatment of asthma (budesonide, beclomethasone dipropionate, flunisolide, fluticasone propionate and triamcinolone acetonide), fluticasone propionate (FP) was the most potent inhibitor of AP-1 and NF-κB activities [12]. These data were in good accordance with the medical practice, with FP being the most potent available inhaled GC

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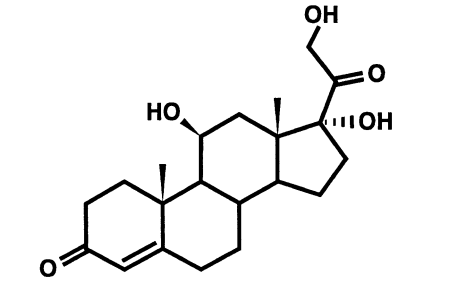
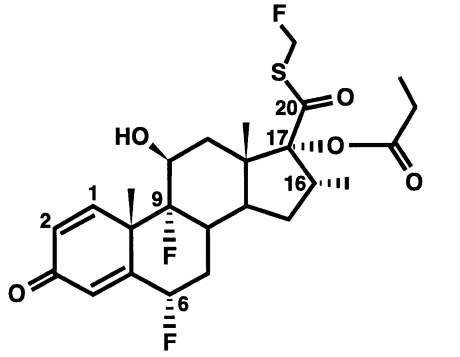
at the time of the study [13]. Mometasone furoate (MF) is a recently developed topical GC. It is currently used for the treatment of rhinitis and is being evaluated as an inhaled formulation for the treatment of asthma. FP and MF possess an esterified lipophilic group in the 17α position (propionate in fluticasone and furoate in mometasone) (Fig. 1), which increases affinity for GRα, uptake and dwell time in tissue on local application, and hepatic first-pass inactivation [14]. Despite their low systemic bioavailability, effects are observed on inflammatory parameters in peripheral blood with FP [15] and on the hypothalamic–pituitary–adrenal (HPA) axis with both molecules [16] after inhalation of high doses. Comparative studies in patients with moderate persistent asthma [17] and perennial rhinitis [18] have shown that MF provides similar clinical improvements as FP. Separate studies have reported that inhaled FP and MF reduce oral GC requirements [19, 20].

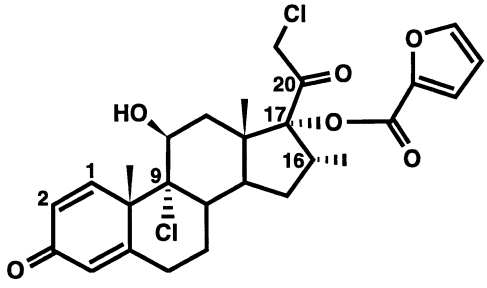
Previous reports have compared the ability of FP and MF to reduce expression of inflammatory markers *in vitro* [21, 22]. However, the relative capacities of FP and MF to repress AP-1 and NF-κB activities have not been directly assessed. In the present work, these were measured using reporter gene assays in transiently transfected A549 lung epithelial cells. In this cell line, we also evaluated the ability of FP and MF to inhibit the endogenous production of Regulated upon Activation, Normal T cell Expressed and Secreted (RANTES), a pro-inflammatory cytokine, whose gene is controlled by AP-1 and NF-κB [23]. Comparison of the transactivation potencies of FP and MF has given unclear results [24]. Therefore, these were assessed here using HeLa cells stably transfected with a GC-inducible luciferase gene. Areas under the dose–response curve (AUCs) were calculated to determine relative potencies. We also measured the maximal effect obtained with either molecule and the concentration causing 50% of this effect (EC50). Finally, we analysed the transcriptional effects of FP and MF at $2 \times 10^{-10}$ M, which is the maximal concentration reached in the plasma after inhalation of high dosages [16, 25].




Cortisol




Fluticasone propionate (FP)




Mometasone furoate (MF)

**Fig. 1.** Structure of cortisol, fluticasone propionate and mometasone furoate. Carbon position numbers in the steroid skeleton where chemical modifications have been introduced are indicated.

## Methods

### *Reagents*

Transferrin-polylysine, poly-L-lysine and spermine were purchased from Sigma (St Louis, MO, USA). TNF-α was purchased from BD Pharmingen (San Diego, CA, USA). Luciferin and dithiothreitol (DTT) were obtained respectively from Euromedex (Souffelweyersheim, France) and Promega (Madison, WI, USA). Coenzyme A was purchased from Boehringer Mannheim (Mannheim, Germany). FP and MF were supplied by GlaxoSmithKline (Greenford, UK) and were initially dissolved in dimethylformamide at $10^{-2}$ M. Further dilutions with a medium containing 5% charcoal/dextran treated fetal calf serum (FCS) were freshly made from original stocks at concentrations ranging from $10^{-14}$ to $10^{-6}$ M. The vials used for the dilutions were pre-incubated with the assay medium to reduce potential sorption of FP and MF to the plastic wall.

### *Plasmids*

The luciferase reporter construct 5xTRE TATA Luc, containing five copies of the AP-1 binding site (TRE) from the collagenase gene upstream of a TATA element, was given by Peter Herrlich (Institute of Genetics, Karlsruhe, Germany). The 3 Igκ Cona Luc plasmid, which contains three tandem repeats of an NF-κB response element (NF-κBRE) from the immunoglobulin κ chain linked to the conalbumin minimal promoter and luciferase gene, was obtained from Alain Israël (Institut Pasteur, Paris, France). The pJ7-*LacZ* plasmid consists of a constitutive promoter (SV40 early promoter) driving the β-galactosidase gene. The c-Fos expression vector pCIFos and the corresponding empty vector pCI (Promega) were provided by Dany Chalbos (Institut National de la Santé et de la Recherche Médicale U540, Montpellier, France). The expression vector for the p65 NF-κB subunit (pECEp65) and the corresponding empty vector (pECE) were given by Carl Scheidereit (Max Delbrük Centre for Molecular Medicine, Berlin, Germany).

### *Cell culture*

HeLa-MMTV-Luc cells [12] and A549 lung epithelial cells were maintained in DMEM/Ham's F12 medium containing 10% heat-inactivated FCS, 100 U/mL penicillin, 100 µg/mL streptomycin and 2 mM glutamine. The day before transfection and/or stimulation, cells were seeded in a medium containing 5% charcoal/dextran-treated FCS.

### *Luciferase assay in HeLa-MMTV-Luc cells*

HeLa-MMTV-Luc cells were seeded at 14 000 cells/well in 96-well microtiter plates. Next day, the cells were treated with GCs at various concentrations. Luciferase assay was performed 24 h later on living cells as described previously [12].

### *Transient transfection of A549 cells and reporter gene assays*

Cells were seeded into 48-well cluster plates. Next day, these were transiently transfected with DNA complexed to replication-deficient adenovirus, transferrin-polylysine and polylysine as described previously [26]. DNA to be transfected included 60 ng/well of the reporter plasmid $3 \times$ Igκ Cona Luc or 5xTRE TATA Luc, 25 ng/well of pJ7-*LacZ* for normalization of transfection efficiency and, when specified, 100 ng/well of the expression vector pCIFos or pECEp65. The corresponding empty vectors pCI or pECE were added to keep the total amount of DNA and promoter constant. After transfection, cells were treated as indicated in the figure legends. To measure AP-1 and NF-κB activities, luciferase activity present in cell extracts was quantified and normalized to β-galactosidase activity as described previously [26].

### *RANTES immunoassay*

A549 cells were seeded into 48-well cluster plates. Next day, these were stimulated for 20 h with 10 ng/mL of TNF-α in combination with FP and MF at various concentrations. Quantification of RANTES release was carried out by ELISA (R&D Systems, Minneapolis, MN, USA).

### *Statistical analysis*

AUCs were calculated using the linear trapezoidal rule. AUCs and values obtained in the different assays were compared using parametric or non-parametric tests depending on the distribution of the data. Statistical significance was set up at $P<0.01$.

## Results

### *Transactivation potency*

Transactivation potency of FP and MF was measured in HeLa-MMTV-Luc cells which carry a GC-inducible promoter linked to the luciferase gene. Dose–response analyses show that FP and MF have identical transactivation potencies (Fig. 2). Indeed, AUCs, $EC_{50}$s and maximal effects are very similar for both molecules (Table 1). At $2 \times 10^{-10}$ M, MF and FP produce a 17-fold induction of promoter activity.

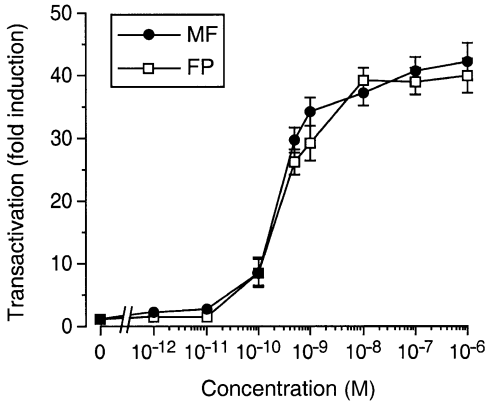



**Fig. 2.** Transactivating effect of MF and FP. HeLa-MMTV-Luc cells which contain a GC-inducible luciferase gene were stimulated with various concentrations of MF or FP for 24 h. Transactivation was then measured by a luciferase assay. Data are shown as fold induction over basal activity and represent the mean ± SEM of five independent experiments performed in triplicates.

**Table 1.** Transactivation potencies of FP and MF

| | AUC (arbitrary units) | $EC_{50}$* (pM) | Maximal effect (fold induction) |
|---|---|---|---|
| FP | 139 (121–157)† | 300 | 40 |
| MF | 146 (126–166) | 300 | 42 |

AUC = area under the dose–response curve; $EC_{50}$ = concentration causing 50% of the maximal effect; FP = fluticasone propionate; MF = mometasone furoate.
*$EC_{50}$s were determined graphically from Fig. 2.
†Mean (95% CI).

### *Transrepression of AP-1 activity*

To measure the effects of MF and FP on AP-1 activity, A549 cells were transiently transfected with the AP-1-dependent reporter plasmid 5xTRE TATA Luc. TNF-α stimulates AP-1 activity only weakly in these cells (Fig. 3a). To induce higher levels of AP-1 activity, the AP-1 component c-Fos was overexpressed by transfection (Fig. 3a). We then tested the capacity of MF and FP to decrease c-Fos-induced AP-1 activity (Fig. 3b). Inhibitory potencies of FP and MF are equivalent as shown by the similar values found for AUCs, $EC_{50}$s and maximal effects (Table 2). Nevertheless, FP has a slightly stronger transrepressive effect than MF at $10^{-7}$ and $10^{-6}$ M ($p<0.01$ with the Mann–Whitney test). At $2 \times 10^{-10}$ M, both GCs inhibit AP-1 activity by 46%.

### *Transrepression of NF-κB activity*

To measure the effects of MF and FP on NF-κB activity, A549 cells were transiently transfected with the NF-κB-dependent reporter plasmid 3 Igκ Cona Luc. NF-κB activity is markedly induced either by TNF-α treatment or overexpression of the NF-κB p65 subunit (Fig. 4a). We show that MF and FP repress p65-induced NF-κB activity in a dose-dependent manner (Fig. 4b). Inhibitory potencies of FP and MF are equivalent as shown by the similar values found for AUCs, $EC_{50}$s and maximal effects (Table 2). At $2 \times 10^{-10}$ M,

FP and MF inhibit NF-κB activity by 47% and 35%, respectively.

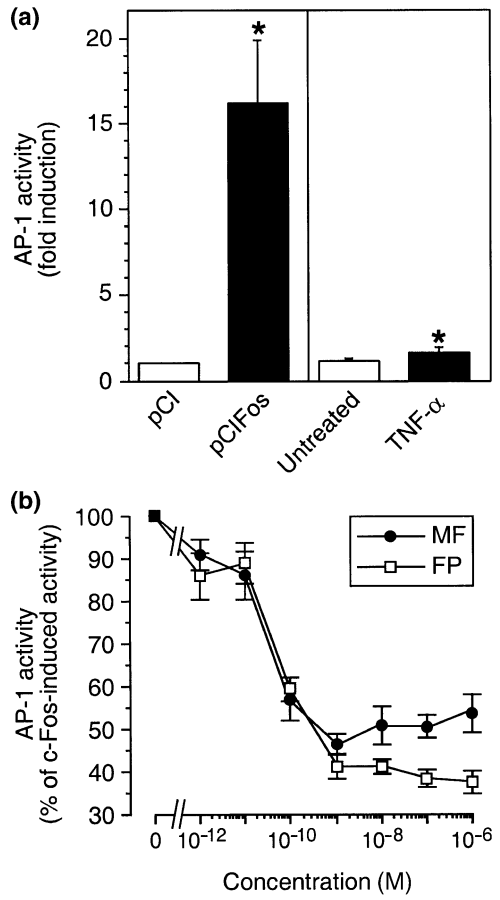



**Fig. 3.** (a) Induction of AP-1 activity by overexpression of the AP-1 component c-Fos or TNF-α treatment. A549 cells were co-transfected with the AP-1-dependent reporter construct 5xTRE TATA Luc, the constitutive β-galactosidase reporter plasmid pJ7-*LacZ* and either the c-Fos expression vector (pCIFos) or the corresponding empty vector (pCI). Alternatively, cells were transfected with the reporter plasmids solely and treated or not with 30 ng/mL TNF-α. Twenty hours post-transfection, the ratio of luciferase activity over β-galactosidase activity was determined to measure AP-1 activity. Data are the mean ± SEM of three independent experiments performed in duplicates and are presented in fold induction. *$P < 0.01$ vs. control with the Mann–Whitney test. (b) Dose-dependent repression of c-Fos-induced AP-1 activity by MF and FP. A549 cells were co-transfected with the plasmids 5 × TRE TATA Luc, pJ7-*LacZ* and pCIFos. Cells were then treated with increasing concentrations of MF or FP for 20 h. AP-1 activity was measured as described above. The c-Fos-induced AP-1 activity was given the nominal value of 100%. Data are shown as percentage and represent the mean ± SEM of three independent experiments performed in triplicates.

### *Inhibition of RANTES production*

The experiments mentioned above were conducted using transfected and artificial promoter-gene constructs. We next assessed the capacity of FP and MF to inhibit the expression of an endogenous gene controlled by AP-1 and NF-κB, namely the RANTES gene. Upon stimulation of A549 cells with TNF-α, RANTES release rises from 9 ± 2 to 3007 ± 133 pg/mL (Fig. 5a). This latter concentration was given the nominal value of 100%. Co-treatment with GCs decreases the production of RANTES in a dose-dependent manner (Fig. 5b). Potencies of FP and MF are equivalent as shown by the similar values found for AUCs, $EC_{50}$s and maximal effects (Table 2). At $2 \times 10^{-10}$ M, MF and FP inhibit RANTES release by 45%.

## Discussion

In the present work, we have compared the transcriptional potencies of FP and MF using reporter gene assays. Dose–response analyses were performed to determine AUCs and $EC_{50}$s. As opposed to the $EC_{50}$, AUC takes into account the effect of the compound over the whole concentration range and is therefore a more accurate measure of the various potencies.

We found that MF and FP are equipotent for transactivation. In a study performed by Smith and Kreutner [24], MF appears to be more potent than FP for transactivation according to $EC_{50}$s, whereas the opposite conclusion is drawn when one considers AUCs. In their study, unusual biphasic curves were obtained, the GRα was overexpressed by transient transfection and no control reporter gene was used to correct for variation in transfection efficiency. Therefore, the discrepant data obtained by Smith and Kreutner may represent variations in the amount of transfected GC-inducible reporter construct or GRα expression vector rather than true differences between GCs. This problem is avoided when the efficiency of transient transfection is assessed or when stably transfected cells are used as in the present work.

A comparison of AUCs indicates that MF and FP have globally the same capacity to repress AP-1 and NF-κB activities. This is consistent with the data obtained on inflammatory mediators whose expression depends at least partly on AP-1 and NF-κB. Indeed, both drugs are equipotent in inhibiting the expression of VCAM-1 [21], IL-4, IL-5 [22] and RANTES (present study). Nevertheless, when data are analysed at a given concentration, FP appears

**Table 2.** Transrepression potencies of FP and MF

| | Inhibition of AP-1 activity | | | Inhibition of NF-κB activity | | | Inhibition of RANTES release | | |
|---|---|---|---|---|---|---|---|---|---|
| | AUC (arbitrary units) | $EC_{50}$* (pM) | Maximal effect (% of inhibition) | AUC (arbitrary units) | $EC_{50}$ (pM) | Maximal effect (% of inhibition) | AUC (arbitrary units) | $EC_{50}$ (pM) | Maximal effect (% of inhibition) |
| FP | 330 (312–348)† | 40 | 63 | 494 (446–542) | 0.1 | 50 | 293 (276–310) | 100 | 78 |
| MF | 362 (331–393) | 40 | 54 | 545 (488–603) | 0.1 | 46 | 299 (256–343) | 100 | 78 |

AP-1 = activator protein-1; AUC = area under the dose–response curve; $EC_{50}$ = concentration causing 50% of the maximal effect; FP = fluticasone propionate; MF = mometasone furoate.
*$EC_{50}$s were determined graphically from Figs 3–5.
†Mean (95% CI).

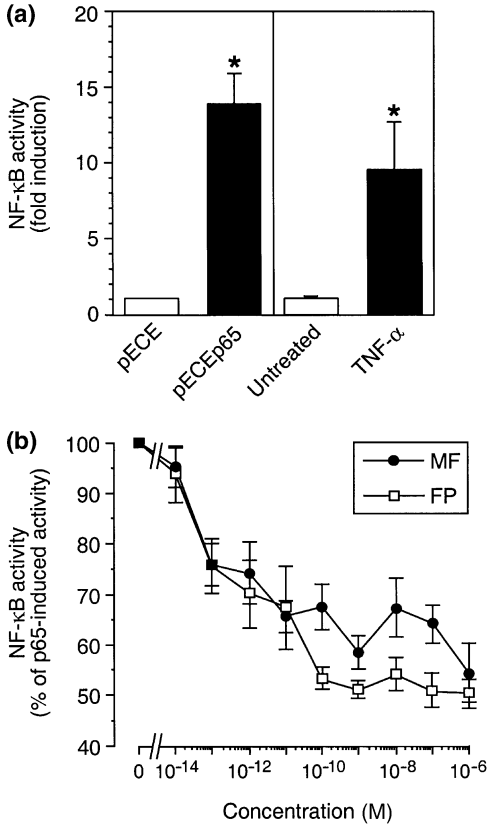



**Fig. 4.** (a) Induction of NF-κB activity by overexpression of the p65 NF-κB subunit or TNF-α treatment. A549 cells were co-transfected with the NF-κB-dependent reporter 3xIgκ Cona Luc, the constitutive β-galactosidase reporter pJ7-*LacZ* and either the p65 expression vector (pECEp65) or the corresponding empty vector (pECE). Alternatively, cells were transfected with the reporter plasmids solely and treated or not with 30 ng/mL TNF-α. Twenty hours post-transfection, the ratio of luciferase activity over β-galactosidase activity was determined to measure NF-κB activity. Data are the mean ± SEM of four independent experiments performed in duplicates and are presented in fold induction. *$P < 0.01$ vs. control with the Mann–Whitney test. (b) Dose-dependent repression of p65-induced NF-κB activity by MF and FP. A549 cells were co-transfected with the plasmids 3 × Igκ Cona Luc, pJ7-*LacZ* and pECEp65. Cells were then cultivated for 20 h in the presence of increasing concentrations of MF or FP. NF-κB activity was measured as described above. The p65-induced activity was given the nominal value of 100%. Data are shown as percentage and represent the mean ± SEM of four independent experiments performed in duplicates.

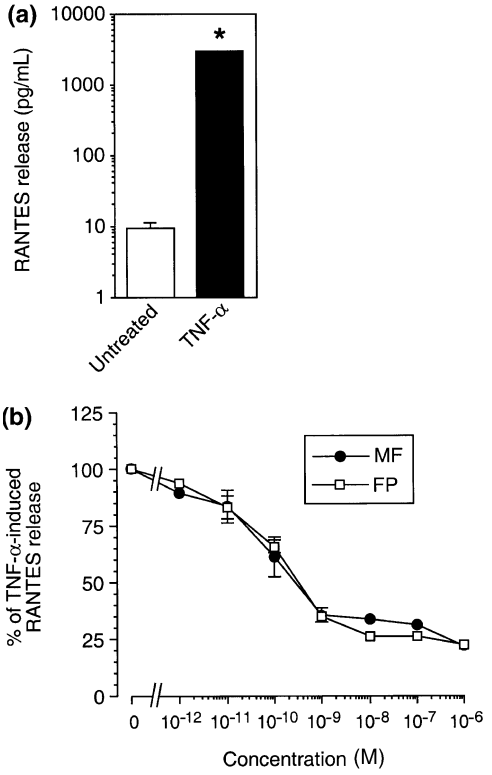



**Fig. 5.** (a) RANTES release upon TNF-α treatment. A549 cells were left untreated or stimulated with 10 ng/mL TNF-α. Twenty hours later, concentration of RANTES in the supernatants was determined using a sandwich enzyme immunoassay. Data represent the mean ± SEM of three independent experiments performed in duplicates. *$P < 0.01$ vs. untreated control with the Mann–Whitney test. (b) Inhibition of RANTES release by MF and FP. A549 cells were stimulated for 20 h with 10 ng/mL TNF-α in the presence of increasing concentrations of MF or FP as indicated. RANTES production was determined using a sandwich enzyme immunoassay. Data are shown as percentage of TNF-α-induced RANTES release and represent the mean ± SEM of three independent experiments performed in duplicates.

to be slightly more potent than MF in repressing AP-1 activity at $10^{-7}$ and $10^{-6}$ M. Although FP may reach such high concentrations in lung tissues after inhalation [27], this small difference does not seem to be clinically relevant. Indeed, MF provides clinical benefit comparable to that observed with FP [17].

Of note, $EC_{50s}$ of FP for transactivation and for repression of NF-κB activity are lower than those found previously with the same assays [12]. In this previous work, potency of FP was probably underestimated. Indeed, FP was dissolved in ethanol and we later realized that FP, as opposed to less lipophilic steroids, could precipitate out of solution when prepared in this solvent. Nevertheless, this does not change the message of this previous study, as FP still appeared as the most potent GC. In the present work, we prepared FP and MF stock solutions in dimethylformamide because dissolution occurs readily and irreversibly in this organic solvent. Also, the vials used for dilutions were pre-incubated with assay medium to reduce potential sorption of FP and MF to the plastic walls.

Although both molecules have low absolute systemic bioavailability as compared to other GCs, their peak plasma concentration after inhalation of relatively high dosages is not negligible. Indeed, this reaches $2 \times 10^{-10}$ M after daily administration of FP 1000 μg or MF 800 μg via dry powder inhaler during 1–4 weeks [16, 25]. At this concentration, FP and MF inhibit RANTES release, and repress AP-1 and NF-κB activities by approximately 40%. Thus, inhaled FP and MF act not only topically on inflammation but also probably in a systemic manner. Although systemic exposure to FP is inefficient in controlling moderate asthma [28], it may prove beneficial in more severe forms of the disease [19]. On the other hand, systemic bioavailability of potent inhaled GCs may be deleterious, particularly in susceptible patients with mild to moderate asthma. For example, FP was found to be equipotent to prednisolone on a milligram potency ratio of 1 : 8.5 for HPA axis suppression [29]. Here we show that FP and MF trigger a very significant transactivating effect at $2 \times 10^{-10}$ M (17-fold induction; see Fig. 2). These data indicate that both compounds may provoke putative

GRE-mediated side-effects (hyperglycaemia, hypertension, oedema, hypokalaemia, glaucoma) and these to the same extent after administration of similar dosages. In this regard, two case reports of asthmatic patients have implicated high doses of FP (1000–2000 μg/day) and budesonide (1200–2000 μg/day) in the loss of diabetic control [30, 31] and in the occurrence of a secondary Cushing's syndrome with hypertension [32]. Whether such adverse effects occur with equivalent doses of MF, as predicted from our analysis, will be determined in the future, as this GC will shortly be available as inhaled formulation. Noteworthy, with lower daily doses of FP (500 μg) or budesonide (800 μg) these effects disappear while asthma control is maintained [30, 32]. This is in accordance with a meta-analysis showing that therapeutic benefit of inhaled FP is achieved with a total daily dose $\leq 500$ μg [33]. Possibly, in asthma patients with a high degree of airway obstruction, the therapeutic index may be set at a higher dose of FP because of reduced systemic bioavailability [34, 35]. Nonetheless, our *in vitro* data support the current trend to prescribe lower doses of the most potent inhaled GCs and to reduce licensed dosage (MF will be licensed up to 800 μg/day whereas FP was licensed up to 2000 μg/day) in order to reach the highest therapeutic ratio. Indeed, as observed with oral GCs [36], we found that transrepression by inhaled GCs occurs at hormone concentrations below those required for transactivation ([12] and present study). Thus, at low concentrations ($\leq 10^{-10}$ M for FP and MF), GCs are expected to have significant anti-inflammatory effects but only weak transactivation-mediated side-effects.

In conclusion, FP and MF have the same ability to trigger gene activation and also the same potency to inhibit AP-1 and NF-κB activities. Their strong positive and negative transcriptional activities at concentration reached in the plasma after inhalation of high doses suggest that these compounds act not only topically but also systemically, with the risk of provoking concomitant adverse effects.

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## References

1 Emorine LJ, Marullo S, Delavier-Klutchko C, Kaveri SV, Durieu-Trautmann O, Strosberg AD. Structure of the gene for human β2-adrenergic receptor: expression and promoter characterization. Proc Natl Acad Sci USA 1987; 84:6995–9.

2 Mak JC, Nishikawa M, Shirasaki H, Miyayasu K, Barnes PJ. Protective effects of a glucocorticoid on downregulation of pulmonary β2-adrenergic receptors *in vivo*. J Clin Invest 1995; 96:99–106.

3 Ming M, Chan W, Wang TT et al. β-Adrenoceptors and dexamethasone synergistically stimulate the expression of the angiotensinogen gene in opossum kidney cells. Kidney Int 1996; 50:94–101.

4 Guo DF, Uno S, Ishihata A, Nakamura N, Inagami T. Identification of a cis-acting glucocorticoid responsive element in the rat angiotensin II type 1A promoter. Circ Res 1995; 77:249–57.

5 Sayegh R, Auerbach SD, Li X et al. Glucocorticoid induction of epithelial sodium channel expression in lung and renal epithelia occurs via *trans*-activation of a hormone response element in the 5′-flanking region of the human epithelial sodium channel α subunit gene. J Biol Chem 1999; 274:12431–7.

6 Jantzen HM, Strahle U, Gloss B et al. Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. Cell 1987; 49:29–38.

7 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; 268:5353–6.

8 Nguyen TD, Chen P, Huang WD, Chen H, Johnson D, Polansky JR. Gene structure and properties of TIGR, an olfactomedin-related glycoprotein cloned from glucocorticoid-induced trabecular meshwork cells. J Biol Chem 1998; 273:6341–50.

9 Cato AC, Wade E. Molecular mechanisms of anti-inflammatory action of glucocorticoids. Bioessays 1996; 18:371–8.

10 Rahman I, MacNee W. Role of transcription factors in inflammatory lung diseases. Thorax 1998; 53:601–12.

11 Göttlicher M, Heck S, Herrlich P. Transcriptional cross-talk, the second mode of steroid hormone receptor action. J Mol Med 1998; 76:480–9.

12 Jaffuel D, Demoly P, Gougat C et al. Transcriptional potencies of inhaled glucocorticoids. Am J Respir Crit Care Med 2000; 162: 57–63.

13 Barnes NC, Hallett C, Harris TA. Clinical experience with fluticasone propionate in asthma: a meta-analysis of efficacy and systemic activity compared with budesonide and beclomethasone dipropionate at half the microgram dose or less. Respir Med 1998; 92:95–104.

14 Brattsand R, Axelsson BI. Basis of airway selectivity of inhaled glucocorticoids. In: Lenfant C, ed. Inhaled glucocorticoid in asthma. New York: Marcel Dekker, 1997:351–80.

15 Bootsma GP, Koenderman L, Dekhuijzen PN, Festen J, Lammers JW, van Herwaarden CL. Effects of fluticasone propionate and beclomethasone dipropionate on parameters of inflammation in peripheral blood of patients with asthma. Allergy 1998; 53:653–61.

16 Affrime MB, Kosoglou T, Thonoor CM, Flannery BE, Herron JM. Mometasone furoate has minimal effects on the hypothalamic–pituitary–adrenal axis when delivered at high doses. Chest 2000; 118:1538–46.

17 O'Connor B, Bonnaud G, Haahtela T et al. Dose-ranging study of mometasone furoate dry powder inhaler in the treatment of moderate persistent asthma using fluticasone propionate as an active comparator. Ann Allergy Asthma Immunol 2001; 86: 397–404.

18 Mandl M, Nolop K, Lutsky BN. Comparison of once daily mometasone furoate (Nasonex) and fluticasone propionate aqueous nasal sprays for the treatment of perennial rhinitis. 194-079 Study Group. Ann Allergy Asthma Immunol 1995; 79:370–8.

19 Noonan M, Chervinsky P, Busse W. Fluticasone propionate reduces oral prednisone use while it improves asthma control and quality of life. Am J Respir Crit Care Med 1995; 152:1467–73.

20 Fish JE, Karpel JP, Craig TJ et al. Inhaled mometasone furoate reduces oral prednisone requirements while improving respiratory function and health-related quality of life in patients with severe persistent asthma. J Allergy Clin Immunol 2000; 106:852–60.

21 Stellato C, Atsuta J, Bickel CA, Schleimer RP. An *in vitro* comparison of commonly used topical glucocorticoid preparations. J Allergy Clin Immunol 1999; 104:623–9.

22 Umland SP, Nahrebne DK, Razac S et al. 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 1997; 100:511–9.
23 Nelson PJ, Kim HT, Manning WC, Goralski TJ, Krensky AM. Genomic organization and transcriptional regulation of the RANTES chemokine gene. J Immunol 1993; 151:2601–12.
24 Smith CL, Kreutner W. *In vitro* glucocorticoid receptor binding and transcriptional activation by topically active glucocorticoids. Arzneimittelforschung 1998; 48:956–60.
25 Falcoz C, Horton J, Mackie AE, Harding SM, Daley-Yates PT. Pharmacokinetics of fluticasone propionate inhaled via the Diskhaler and Diskus powder devices in patients with mild-to-moderate asthma. Clin Pharmacokinet 2000; 1:31–7.
26 Mathieu M, Gougat C, Jaffuel D et al. The glucocorticoid receptor gene as a candidate for gene therapy in asthma. Gene Ther 1999; 6:245–52.
27 Esmailpour N, Hogger P, Rabe KF, Heitmann U, Nakashima M, Rohdewald P. Distribution of inhaled fluticasone propionate between human lung tissue and serum *in vivo*. Eur Respir J 1997; 10:1496–9.
28 Lawrence M, Wolfe J, Webb DR et al. Efficacy of inhaled fluticasone propionate in asthma results from topical and not from systemic activity. Am J Respir Crit Care Med 1997; 156:744–51.
29 Wilson AM, Lipworth BJ. Short-term dose–response relationships for the relative systemic effects of oral prednisolone and inhaled fluticasone in asthmatic adults. Br J Clin Pharmacol 1999; 48: 579–85.
30 Faul JL, Tormey W, Tormey V, Burke C. High dose inhaled corticosteroids and dose dependent loss of diabetic control. BMJ 1998; 317:1491.
31 Faul JL, Cormican LJ, Tormey VJ, Tormey WP, Burke CM. Deteriorating diabetic control associated with high-dose inhaled budesonide. Eur Respir J 1999; 14:242–3.
32 Wilson AM, Blumsohn A, Jung RT, Lipworth BJ. Asthma and Cushing's syndrome. Chest 2000; 117:593–4.
33 Holt S, Suder A, Weatherall M, Cheng S, Shirtcliffe P, Beasley R. Dose–response relation of inhaled fluticasone propionate in adolescents and adults with asthma: meta-analysis. BMJ 2001; 323:253–6.
34 Weiner P, Berar-Yanay N, Davidovich A, Magadle R. Nocturnal cortisol secretion in asthmatic patients after inhalation of fluticasone propionate. Chest 1999; 116:931–4.
35 Brutsche MH, Brutsche IC, Munawar M et al. Comparison of pharmacokinetics and systemic effects of inhaled fluticasone propionate in patients with asthma and healthy volunteers: a randomised crossover study. Lancet 2000; 356: 556–61.
36 Jonat C, Rahmsdorf HJ, Park K-K et al. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell 1990; 62: 1189–204.