The impact of drug clearance on duration of action of corticosteroids

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Summary

Corticosteroids (CS) are widely used in the treatment of asthma and chronic obstructive pulmonary disease. Following inhalation, corticosteroids are subjected to drug clearance within the lungs. Their duration of action is dictated by their physiochemical properties, which subsequently impacts on their binding and activation of the glucocorticoid receptor (GR), essential for corticosteroid function. Upon activation, GR translocates into the nucleus, where it reduces expression of pro-inflammatory markers, such as CXCL8. We hypothesised that fluticasone furoate (FF), a once-daily CS that has superior affinity and a slower rate of dissociation from GR than budesonide (BUD) and fluticasone propionate (FP) will maintain its action and function following a 20h washout period. The effects of BUD, FP and FF on GR nuclear translocation over a 24h time-course was examined in U937 monocytes. Importantly, we compared the effects of a 20h washout on GR nuclear localisation and suppression of CXCL8 following treatment with CS for 4h. Statistical analysis was performed using non-parametric analyses and results presented as mean±SEM. Treatment with BUD, FP and FF significantly induced GR nuclear translocation at 24h. Importantly, a 20h washout following a 4h treatment period reduced BUD- and FP-induced GR nuclear localisation to basal levels, whereas FF maintained similar levels of GR nuclear translocation. A corresponding difference in CXCL8 suppression was also observed following the 20h washout period. Exposure of cells to FF for 4h (followed by 20h washout) was as effective as continued exposure for 24h.

Introduction

Corticosteroids (CS) are widely used in the treatment of asthma as monotherapy and, in both asthma and chronic obstructive pulmonary disease (COPD) in combination with bronchodilators (1). Following inhalation, corticosteroids are subjected to drug clearance within the lungs. Mechanisms that affect drug clearance include airflow geometry and velocity, humidity and mucociliary clearance (2). The duration of action of CS is dictated by their physiochemical properties, which subsequently impacts on their binding and activation of the glucocorticoid receptor (GR), essential for corticosteroid function (3). Briefly, CS exert their anti-inflammatory effects by binding and activation of GR. Upon activation, GR translocates into the nucleus, where it either upregulates transcription of anti-inflammatory genes or downregulates the transcription of pro-inflammatory genes such as CXCL8 (4).

Better understanding of the structure activity relationships of CS has led to the development of new CS with higher therapeutic ratios and lower systemic bioavailabilities. Fluticasone propionate (FP) has been shown to have high affinity and selectivity for GR and low systemic absorption. GR affinity of FP and mometasone furoate (MF) is estimated to be about 12 and 5 times of dexamethasone and BUD, respectively (5). More recently, fluticasone furoate (FF), a novel longer-acting CS, developed as a once-daily CS for asthma and COPD, has been shown to have a greater affinity for GR than FP. Valotis and Hogger have shown, FF has a very fast association and a slow dissociation from human GR binding kinetics, with a relative receptor affinity (RRA; relative to dexamethasone (RRA: 100)) of 2,988, in comparison with other corticosteroids, which have a much lower receptor affinity: MF, 2,244; FP, 1775; and BUD, 855 (6). This may be partly due to better H-bonding and van der Waals interaction of the 17-α ester group of FF with the ligand-binding site of GR, giving rise to a prolonged duration of action as seen in dose ranging studies in asthmatics, where FF had at least 23h duration of efficacy (7, 8). Based on these findings we proposed that FF should achieve a similar level of GR nuclear translocation following a 20h washout period as seen with 24h continual treatment.

Experimental Methods

U937 monocytes are a cell suspension cell line, which are commonly used to investigate the anti-inflammatory effects of CS and their molecular pathways. U937 monocytes serve as an *in vitro* model for monocyte/macrophage differentiation. Macrophages are considered an important pathogenic cell in asthma and COPD patients. The effects of BUD, FP and FF on GR nuclear translocation over a 24h time-course was examined in U937 monocytes. In addition, we compared the effects of a 20h washout period on GR nuclear localisation and suppression of CXCL8 following treatment with CS for 4h. In the drug washout experiments, drugs were incubated for 4h to ensure corticosteroid-induced GR nuclear translocation was at maximal levels before drugs were removed. Nuclear and cytoplasmic fractions were extracted and levels of GR were detected by Western blotting. In addition, U937 monocyte cells were stimulated with IL-1β (10 ng/ml) followed by treatment with CS (BUD, FP and FF) for 24h in the presence and absence of a 20h washout period. Pro-inflammatory mediator CXCL8 (ng/ml) was measured in cell supernatant using ELISA. Statistical analysis was performed using non-parametric analyses and results presented as mean±SEM.

Results

Continual exposure for 24h to BUD, FP and FF at 10^{-9}M significantly induced GR nuclear localisation by 3.0±0.61, 5.6±0.88 and 8.3±0.84-fold, respectively (*p<0.05 compared to unstimulated controls) (Fig 1). FF-induced GR nuclear localisation was significantly greater than BUD, but comparable to that achieved with FP. In the 20h washout experiments, there was a similar level of GR nuclear translocation seen with FF (10^{-9}M) treatment at 24h (4+20h) as seen with continual FF exposure (5.4±1.0- versus 6.3±0.84-fold increase, p=ns) (Fig 2). Although, FP- and BUD-induced GR nuclear translocation were dramatically reduced back to basal levels in the 20h washout model. A corresponding difference in CXCL8 suppression was also observed (Fig 3).

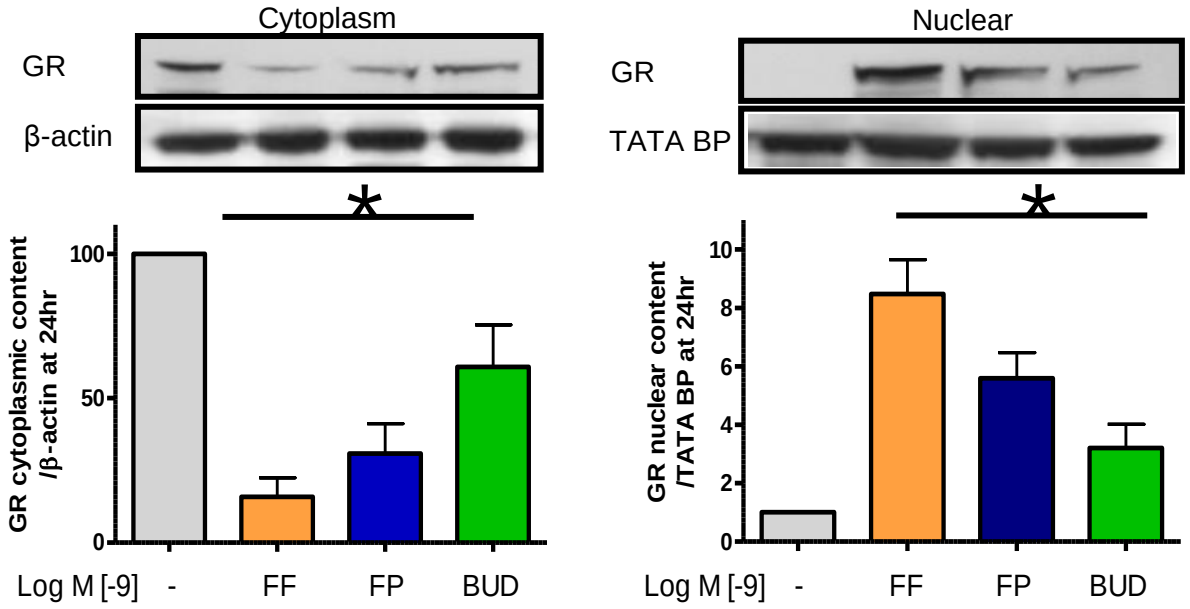

Fig 1 Effect of budesonide (BUD), fluticasone propionate (FP) and fluticasone furoate (FF), all at 10^{-9}M, on glucocorticoid receptor (GR) nuclear translocation, as determined by cytoplasmic and nuclear GR levels. Results represent the mean±SEM of 3 independent experiments. *p<0.05 vs. basal.

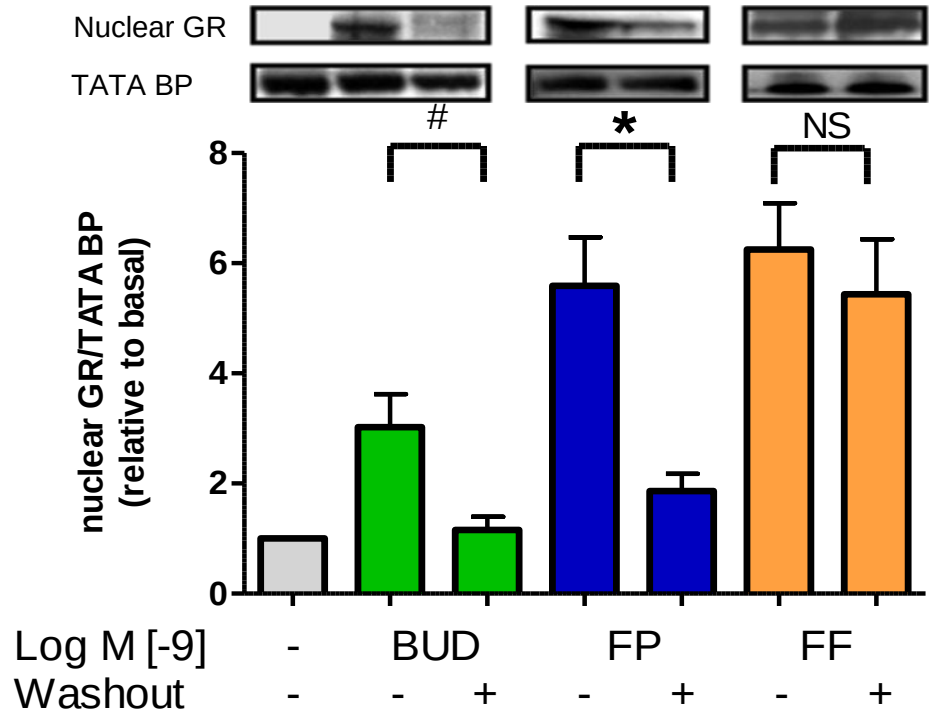

Fig. 2 Effects of budesonide (BUD), fluticasone propionate (FP) and fluticasone furoate (FF) on GR nuclear localisation. Results represent the mean±SEM of a minimum of 3 independent experiments. #p<0.05 vs. 24h BUD 10^{-9}M contact. *p<0.05 *vs*. 24h FP 10^{-9}M contact.

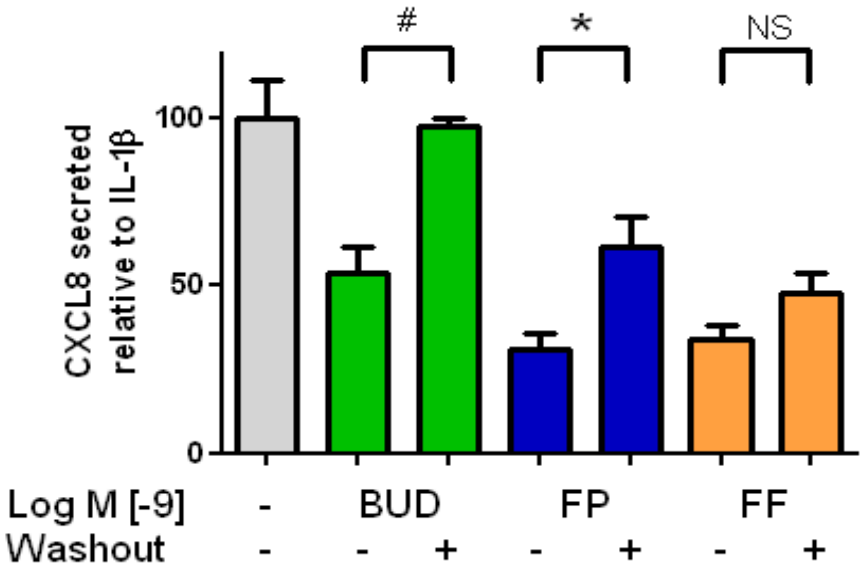

Fig 3 Effects of budesonide (BUD), fluticasone propionate (FP) and fluticasone furoate (FF) on IL-1β-induced CXCL8. Results represent the mean±SEM of a minimum of 3 independent experiments. #p<0.05 vs. 24h BUD 10^{-9}M contact. *p<0.05 vs. 24h FP 10^{-9}M contact.

Conclusion

In the 24h drug contact experiments, FF-induced GR nuclear translocation was far greater than FP and BUD. This reflects the greater binding affinity of FF for GR compared to FP and BUD. In the 20h drug washout experiments, following 4h corticosteroid incubation, FF not only maintained its level of GR nuclear localisation and suppression of IL-1β-induced CXCL8, it was also far superior to both BUD and FP. In contrast, BUD- and FP-induced GR nuclear translocation were significantly reduced to basal levels following a 20h washout. In addition, BUD-mediated suppression of IL-1β-induced CXCL8 was completely abolished and FP-mediated suppression of IL-1β-induced CXCL8 was reduced significantly. The longer duration of action of FF in the drug washout experiment can be explained by its enhanced affinity for GR and slower rate of dissociation. Our *in vitro* washout model can distinguish between corticosteroids with different durations of action and binding affinities for GR, the intracellular target of corticosteroids. Importantly, our *in vitro* model can determine which corticosteroids may continue to provide 24h efficacy and consequently clinical benefit as a once-daily therapy.

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