Potential mechanisms to explain how LABAs and PDE4 inhibitors enhance the clinical efficacy of glucocorticoids in inflammatory lung diseases

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Abstract

Inhaled glucocorticoids acting via the glucocorticoid receptor are a mainstay treatment option for individuals with asthma. There is a consensus that the remedial actions of inhaled glucocorticoids are due to their ability to suppress inflammation by modulating gene expression. While inhaled glucocorticoids are generally effective in asthma, there are subjects with moderate-to-severe disease in whom inhaled glucocorticoids fail to provide adequate control. For these individuals, asthma guidelines recommend that a long-acting β₂-adrenoceptor agonist (LABA) be administered concurrently with an inhaled glucocorticoid. This so-called “combination therapy” is often effective and clinically superior to the inhaled glucocorticoid alone, irrespective of dose. LABAs, and another class of drug known as phosphodiesterase 4 (PDE4) inhibitors, may also enhance the efficacy of inhaled glucocorticoids in chronic obstructive pulmonary disease (COPD). In both conditions, these drugs are believed to work by elevating the concentration of cyclic adenosine-3’,5’-monophosphate (cAMP) in target cells and tissues. Despite the success of inhaled glucocorticoid/LABA combination therapy, it remains unclear how an increase in cAMP enhances the clinical efficacy of an inhaled glucocorticoid. In this report, we provide a state-of-the-art appraisal, including unresolved and controversial issues, of how cAMP-elevating drugs and inhaled glucocorticoids interact at a molecular level to deliver enhanced anti-inflammatory benefit over inhaled glucocorticoid monotherapy. We also speculate on ways to further exploit this desirable interaction. Critical discussion of how these two drug classes regulate gene transcription, often in a synergistic manner, is a particular focus. Indeed, because interplay between glucocorticoid receptor and cAMP signaling pathways may contribute to the superiority of inhaled glucocorticoid/LABA combination therapy, understanding this interaction may provide a logical framework to rationally design these multicomponent therapeutics that was not previously possible.

Introduction

Asthma is a complex inflammatory disorder of the airways and lungs for which inhaled glucocorticoids – commonly referred to as corticosteroids or simply steroids – are a recommended treatment option (www.ginasthma.org). Most patients with asthma are responsive to the remedial actions of inhaled glucocorticoids. However, a proportion of individuals with moderate-to-severe disease, in whom inflammation is pronounced, are not effectively managed by inhaled glucocorticoids regardless of dose. In these cases, asthma guidelines recommend that a LABA be administered concurrently with an inhaled glucocorticoid as a combination therapy [1,2]. This often provides asthma control and is clinically superior to the inhaled...
glucocorticoid alone using a variety of outcome measures, including lung function, symptoms, the need for rescue medication and the frequency of exacerbations [3–5]. Inhaled glucocorticoid/LABA combination therapy given in a single inhaler device has been extremely successful, with Advair®/Seretide® (fluticasone propionate plus salmeterol xinafoate) being ranked third in 2010 in the top 10 drugs based on sales [6]. This success has fuelled the development of second generation inhaled glucocorticoid/LABA combination therapy, which has a longer duration of action suitable for once-a-day dosing that may translate into improved patient compliance and, hence, asthma control [7]. Inflammation of the Airways combined with systemic inflammation is also a cardinal feature of COPD, and there is evidence that in certain patient populations inhaled glucocorticoid/LABA combination therapy is clinically superior to both LABA and inhaled glucocorticoid monotherapy [8–11]. Indeed, patients of a severe bronchitic phenotype, who have pronounced inflammation, are most responsive to this intervention using several metrics, including frequency of hospitalizations, exacerbation rate, inflammatory markers, lung function, and quality of life [8–12]. Additional clinical benefit may also be produced by the PDE4 inhibitor, roflumilast, when combined with an inhaled glucocorticoid in patients with COPD of the same phenotype [13].

The mechanism(s) underlying the superiority of these multicomponent therapeutics is unknown. A widely held view is that a LABA, in addition to promoting long-lasting bronchodilatation, enhances the anti-inflammatory actions of an inhaled glucocorticoid in a cAMP-dependent manner [2,14–16]. PDE4 inhibitors are likely to work in a similar way [17]. It is believed that an inhaled glucocorticoid acting via the glucocorticoid receptor suppresses inflammation by modifying gene expression [16]. Two general paradigms have been proposed. One of these is called transrepression, where the glucocorticoid receptor binds to and inhibits the activity of transcription factors responsible for activating pro-inflammatory genes. The other process is transactivation, in which the glucocorticoid receptor promotes the transcription of anti-inflammatory genes [16,18–23]. Despite inhaled glucocorticoid/LABA combination therapy being available since 1998, unresolved and controversial issues remain. How activation of the β2-adrenoceptor on pro-inflammatory and immune cells in the lung augments glucocorticoid action and whether anything else can be done to further enhance clinical efficacy are particularly important areas of research. Certainly, β2-adrenoceptor agonists produce several, potentially unwanted, effects, including pro-inflammatory cytokine production, which are reduced or prevented by a glucocorticoid [2,16]. Equally, glucocorticoids can increase β2-adrenoceptor expression and arrest desensitization, which should preserve the beneficial actions of a LABA [2,16]. However, while these interactions are demonstrable in isolated cells and tissues, their clinical relevance is unclear and they are unlikely to explain the superiority of inhaled glucocorticoid/LABA combination therapy in asthma and COPD. In this report, we focus on the probability that cAMP directly enhances the anti-inflammatory activity of an inhaled glucocorticoid by up-regulating glucocorticoid receptor-mediated signaling leading to improved clinical outcomes. This tenet is consistent with clinical data showing improved therapeutic activity when pulmonary co-deposition of both drugs is maximised (vide infra).

**How does a LABA enhance the clinical effects of an inhaled glucocorticoid?**

Conceptually, two mutually inclusive possibilities could explain the clinical superiority of inhaled glucocorticoid/LABA combination therapy. Simplistically, a LABA and a glucocorticoid may act in a purely additive manner by exerting a variety of independent, non-interacting responses that may or may not be sensitive to either drug alone (Figure 1A). The induction of DUSP1 (dual specificity phosphatase 1), a putative anti-inflammatory gene encoding a phosphatase responsible for inactivating mitogen-activated protein kinases that are typically "switched on" by pro-inflammatory stimuli [24], is a good example [25,26] (Figure 1A). However, while simple additive interactions undoubtedly occur, the clinical data also accommodate the idea of synergy between the glucocorticoid receptor and cAMP signaling pathways (Figure 1B). For example, RGS2 (regulator of G-protein signaling 2), like DUSP1, is a gene whose expression in airway smooth muscle and epithelial cells is induced by a LABA [27,28] and, to a lesser degree, a glucocorticoid [27–30]. However, unlike DUSP1, RGS2 expression is increased synergistically (defined here as a response produced by two drugs in a combination that is greater than the sum of their individual effects) when these agents are used concurrently [27] (Figure 1B). RGS2 is a GTPase-activating protein that terminates agonist-induced signaling mediated by G-protein-coupled receptors that work through Gq [31,32]. In inflammatory lung diseases, RGS2 exerts effects that may be interpreted as beneficial, such as bronchoprotection and the down-regulation of pro-inflammatory signaling, including those pathways that up-regulate mucus (MUC5AC) production [27,28,33,34]. Other genes that have potential anti-inflammatory activity, such as CD200 and CRISPLD2 [35–38], are also induced in a synergistic manner by a LABA and a glucocorticoid in combination [39], suggesting that this molecular interaction may be commonplace.
Another, mechanistically distinct form of synergy has been demonstrated in cells transfected with luciferase reporters that respond only to glucocorticoids \[25,40\]. In human bronchial epithelial cells and airway myocytes, glucocorticoid receptor-mediated reporter activation is significantly enhanced by a LABA \[25\]. In these same cell types, this effect is also reproduced at the level of gene expression with \textit{CDKN1C}, which encodes a cell cycle kinase inhibitor that could be beneficial in asthma and COPD \[41,42\], being a representative example \[25,43\]. Thus, there are glucocorticoid-induced responses that are up-regulated by a LABA despite the LABA alone being inactive (Figure 1B). Theoretically, the opposite profile could be displayed whereby a glucocorticoid augments...
the effect of a LABA but is inactive itself. Responses that have an absolute requirement for LABA and glucocorticoid are also possible (Figure 1B).

Currently, the molecular mechanism underlying the inhaled glucocorticoid/LABA interaction remains elusive, with multiple, potentially conflicting, ideas having been proposed. In terms of understanding this effect, most studies have focused on the ability of cAMP to enhance glucocorticoid receptor function. Indeed, data from the 1990s show that activation of the cAMP pathway enhances glucocorticoid receptor-mediated transcription [44–47]. However, at that time, there was a lack of clarity regarding the way in which cAMP and glucocorticoid receptors interact at a molecular level. Indeed, evidence for and against the idea that cAMP enhances the binding of the glucocorticoid receptor to DNA is available [44–47]. This controversy has been perpetuated with claims and counter-claims that cAMP-elevating drugs, including LABAs, enhance the translocation of the glucocorticoid receptor to the nucleus [45,48–53]. Variable effects of cAMP on glucocorticoid receptor expression levels and glucocorticoid receptor binding have also added to the confusion [45–47].

In considering how a LABA could enhance glucocorticoid receptor-mediated signaling, an appreciation of possible cell- and system-dependent effects should not be overlooked. Nevertheless, it is clear from data garnered in single cell types (e.g. airway epithelial cells) that some glucocorticoid-induced genes are not affected by a LABA, whereas other genes are enhanced in an additive or often synergistic manner [25]. Such observations correspondingly require explanations that accommodate gene-specific regulation rather than a global modification of glucocorticoid receptor function. For example, the RGS2 promoter has a putative glucocorticoid receptor binding region [54] and functional sites for the cAMP-regulated transcription factor (CREB) [55]. Thus, the concurrent binding of the glucocorticoid receptor and CREB to DNA in a target gene may very well lead to transcriptional synergy. Equally, a number of other transcription factors (e.g. CCAAT-enhancer-binding proteins) can be induced by glucocorticoid and cAMP, ultimately leading to transcriptional synergies at complex glucocorticoid receptor-binding sites in certain gene promoters [40]. Further potential for gene-specific transcriptional regulation is provided by the finding that cAMP modulates the behaviour of obligatory co-activators and/or co-repressors that are known to play roles in transcriptional programming mediated by a variety of nuclear hormone receptors, including the glucocorticoid receptor [56–60]. For example, cAMP augments progesterone receptor-mediated gene expression by a mechanism that involves the phosphorylation and dissociation of nuclear co-repressors 1 and 2, rendering the ligand-bound receptor more transcriptionally competent [60,61]. Similar regulation of the glucocorticoid receptor seems likely.

**The importance of pharmacodynamics**

Inhaled glucocorticoids acting through the glucocorticoid receptor exert a pleiotropic suppressive effect of many cell types that collectively promote the chronic inflammation that characterises asthma and COPD. However, glucocorticoid receptor density varies considerably between different human tissues [62–64] and this has important pharmacodynamic implications. The ability of an inhaled glucocorticoid to produce a response is, in its simplest form, the product of intrinsic efficacy (a sole property of the drug determined by its structure) and the number of functional glucocorticoid receptors in a given target (a tissue-dependent parameter). Therefore, glucocorticoid receptor density is a key determinant of the magnitude of gene transactivation and transrepression that can be produced by a given glucocorticoid [65–67]. Equally, at constant glucocorticoid receptor number in a given cell type, response will also be determined by the structure of the glucocorticoid and whether or not the glucocorticoid receptor forms an optimal or suboptimal conformation to effect transrepression or bind DNA and transactivate a particular gene. In pharmacological terms, these parameters will define a glucocorticoid as a full agonist, partial agonist or even antagonist in a tissue- and response-dependent manner. In considering gene transactivation as a functionally-relevant, anti-inflammatory output, it is clear that the gene expression “fingerprint” produced by different glucocorticoid receptor agonists across a panel of target tissues will not be invariant and could provide a basis for the pharmaceutical industry to rationally design new inhaled glucocorticoid or inhaled glucocorticoid/LABA combination therapy [16]. Clearly, compounds would have to be screened in target and off-target human tissues and the physiological role(s) of genes in the “fingerprint” defined. With this knowledge, glucocorticoids could be identified and selected to preferentially induce genes with anti-inflammatory activity, while at the same time minimising those that mediate unwanted effects.

The ability of a LABA to enhance anti-inflammatory glucocorticoid activity will also be governed by the same pharmacodynamic principles that apply to a glucocorticoid receptor, as well as the efficiency with which the β2-adrenoceptor can generate cAMP. There are ~30,000–40,000 β2-adrenoceptors on a human airway smooth muscle cell [68], whereas, on human neutrophils and T-lymphocytes, functional receptor expression is considerably (30 to 50 fold) lower [69,70]. Logic dictates that the enhancement of anti-inflammatory
glucocorticoid activity by a LABA may be modest, or even absent, in a cell where $\beta_2$-adrenoceptor number is limiting. Weak receptor-adenyl cyclase coupling in a particular pro-inflammatory or immune cell type may also render a LABA with low intrinsic efficacy, such as salmeterol, unable to enhance glucocorticoid activity. For example, although eosinophils express a moderate number of $\beta_2$-adrenoceptors (~4300 sites/cell) [71], their ability to couple to adenyl cyclase is apparently weak, such that salmeterol behaves as an antagonist [72,73]. Based on the aforementioned discussion, it may not be unreasonable to speculate that the relatively poor activity of an inhaled glucocorticoid or an inhaled glucocorticoid/ LABA combination therapy in COPD is due, in part, to the involvement of immune and pro-inflammatory cells that express, intrinsically, a low density of functional glucocorticoid receptors. Similar insensitivity may also result if the $\beta_2$-adrenoceptor population on those same cells is low and/or are coupled inefficiently to adenyl cyclase (vide infra). However, the possibility that exposure of target cells and tissues to cigarette smoke, a primary cause of COPD, reduces the expression of, and signaling mediated by, these receptors is also plausible and should be considered.

**Enhancing the anti-inflammatory effects of glucocorticoids with PDE inhibitors**

While asthma is often controlled with an inhaled glucocorticoid or an inhaled glucocorticoid/LABA combination therapy, COPD is considerably less responsive to the anti-inflammatory actions of these drugs. Accordingly, there is a clear unmet clinical need for more effective therapies. The appreciation of distinct COPD phenotypes [74–77] allows patients to be classified using a variety of criteria, including “response to treatment”. Patients with severe disease, who experience frequent exacerbations and have a history of productive cough or expectoration, represent a COPD phenotype that may respond to PDE4 inhibitors, such as roflumilast (as well as inhaled glucocorticoid/LABA combination therapy). Indeed, patients with this form of COPD often exhibit pronounced inflammation [78], suggesting that they are most likely to respond to anti-inflammatory drugs [79]. Moreover, because LABAs and PDE4 inhibitors act on the same signaling pathway (i.e. they increase cAMP synthesis and block cAMP degradation, respectively), they may act synergistically. Thus, conceptually, the high level of inflammation seen in severe, bronchitic COPD [78] may be more responsive to an inhaled glucocorticoid, LABA and PDE4 inhibitor if used together rather than individually. This assumption, which is being tested in phase IV clinical trials [80], is reflected in current treatment guidelines, which recommend that roflumilast be given to patients already taking an inhaled glucocorticoid/LABA combination therapy (www.goldcopd.org).

In the previous section, we speculated that the inability of a LABA alone to optimally enhance glucocorticoid activity in patients with COPD in whom inflammation is prevalent may be due to a dominant participation of immune and/or pro-inflammatory cell types where $\beta_2$-adrenoceptor number is limiting. If this is true then, theoretically, this insensitivity could be overcome, partially or even totally, with a PDE4 inhibitor in the form of a triple combination therapy [17]. Indeed, a PDE4 inhibitor, by blocking cAMP degradation, might transform a cell that exhibits weak sensitivity to a LABA into one that now generates a cAMP signal of sufficient magnitude to enhance anti-inflammatory glucocorticoid activity above the level produced by an inhaled glucocorticoid/LABA combination therapy alone.

Recently, there has been renewed interest in targeting PDE3, another family of cAMP PDEs, in inflammatory lung diseases [81]. Tight-binding PDE3 inhibitors, such as the highly selective compound RPL554 [82], have been evaluated in clinical trials of asthma and COPD with promising results [83]. Although it is unclear if a PDE3 inhibitor can enhance the anti-inflammatory effects of an inhaled glucocorticoid in the clinical setting (vide infra), it has been shown that combining a PDE3 and PDE4 inhibitor sensitizes human airway epithelial cells to a LABA and extends the duration of glucocorticoid- and LABA-induced gene expression, relative to a PDE4 inhibitor alone [84]. Such data, if reproduced in vivo, might prolong anti-inflammatory activity and give rise to inhaled glucocorticoid/LABA combination therapy being administered concurrently with inhibitors of multiple PDEs.

**Novel combination therapies**

The ability of LABAs and PDE4 inhibitors to enhance glucocorticoid receptor-mediated gene expression is reproduced by several but not all agents that increase cAMP [25,85–89]. Agonists of the EP2-, EP4- and IP prostanoid receptors and the adenosine A2B-receptor subtype all display this property [87,89], whereas inhibitors of PDE2, PDE3 and PDE7 do not [39,90]. Theoretically, a more generic effect of cAMP-elevating drugs on glucocorticoid receptor-mediated gene expression could be exploited to therapeutic advantage in inflammatory diseases where perhaps $\beta_2$-adrenoceptor agonists are poorly effective, due to low functional receptor expression or weak coupling to adenyl cyclase in target cells and tissues. In this context, detailed knowledge of G protein-coupled receptor expression could allow various cAMP-elevating agonists to be “added-on” to an inhaled glucocorticoid, depending on...
the specific inflammatory disease of interest. Such effects could be further enhanced by identifying receptors that may be less prone to agonist-induced desensitization and/or by “adding on” one or more PDE inhibitors.

**Maximising efficacy by ensuring drug co-deposition: development of conjugated pro-drugs and bifunctional ligands**

The ability of a LABA (and PDE4 inhibitor) to augment the anti-inflammatory actions of an inhaled glucocorticoid requires that these drugs reach the same target cells and tissues at concentrations that allow them to interact at a molecular level (*vide supra*). This is not a trivial point. Biophysical analyses have revealed significantly greater pulmonary co-deposition of an inhaled glucocorticoid and LABA when delivered by a combination meter dose inhaler than if each drug is given separately [91]. Moreover, this optimised delivery translates into a significantly greater improvement in lung function [92,93]. Thus, the co-deposition of both components of an inhaled glucocorticoid/LABA combination therapy is necessary if maximum therapeutic benefit is to be realised. Intuitively, this is more likely to occur when both drugs are inhaled in a single breath from a single inhaler device, as this would facilitate the ability of the LABA and inhaled glucocorticoid to interact. However, even this method of delivery is unlikely to provide optimal drug co-deposition, necessitating the development of alternative approaches to achieve this goal. Two possibilities are discussed here: conjugated pro-drugs and bifunctional ligands.

A pro-drug is an inactive or weakly active molecule that typically undergoes enzymatic activation *in vivo* [94]. The patent literature contains several claims for mutual inhaled pro-drugs consisting of a glucocorticoid and a LABA [95,96]. An example is shown in Figure 2 in which salmeterol is conjugated to a derivative of the inhaled glucocorticoid, desisobutyryl-ciclesonide. This particular pro-drug is activated by alkaline phosphatase and an esterase, which are enriched in the lung relative to saliva and plasma. In theory, conjugated pro-drugs of this form would ensure co-deposition and selective activation in the lung with an improved side-effect profile.

In contrast, bifunctional ligands are single chemical entities that contain two pharmacophores joined covalently by an optimally designed “spacer” at, so-called, linker sites (Figure 2) [97,98,99]. Typically, such ligands have a high molecular weight. For an inhaled drug, this often translates into greater lung retention, low oral bioavailability and reduced systemic exposure [98]. Moreover, the clinical development of bifunctional ligands is simplified in terms of matched pharmacokinetics, formulation and, critically, identical deposition characteristics [99]. GS-5759 is a novel, heterobifunctional ligand developed by Gilead Sciences [100] for COPD in which the exceptionally potent PDE4 inhibitor, GlaxoSmithKline (GSK) 256066 [101], is conjugated to the active head group of the LABAs, indacaterol and carmoterol, via a pent-1-y-1yl benzene spacer (Figure 3) [100,102,103]. This molecule has a similar potency at both targets and has been optimised for inhaled delivery with potential as a first-line therapy in COPD or combined with an inhaled glucocorticoid as part of a triple combination therapy within a single inhaler device. Clearly, the cAMP signal generated by concurrent PDE4 inhibition and β2-adrenoceptor activation produced by a single molecule in the same cell over a similar concentration range would allow a greater therapeutic benefit of a glucocorticoid to be realised, especially in target tissues in which β2-adrenoceptor number is limiting.

**Concluding remarks**

The use of inhaled glucocorticoid/LABA combination therapy in the control of asthma and COPD is entrenched in all national and international treatment guidelines. While the mechanism of action of combination therapy is not understood, the balance of evidence supports the idea that the regulation of glucocorticoid-induced transcription by cAMP is gene-specific rather than a global, non-selective effect on the expression of the “glucocorticoid transcriptome”. Pharmacodynamic analyses predict that gene expression will differ (maybe considerably) in both a tissue and glucocorticoid/LABA-dependent manner. While considerable investigation is still necessary to achieve a systematic characterization of these effects, such knowledge will provide significant opportunity to rationally design new inhaled glucocorticoid/LABA combination therapy perhaps with a more optimised, effective and safer gene expression profile. Gene transrepression is also believed to contribute to the remedial actions of glucocorticoids in inflammatory lung diseases. However, the relationship between this process and transactivation remains unclear [19,20] and, currently, there is little direct evidence that a LABA and a glucocorticoid transrepress gene expression in a synergistic manner. Nevertheless, the ability of glucocorticoids to repress inflammatory gene expression can often require glucocorticoid receptor-dependent transactivation. Therefore, under these circumstances, beneficial interactions between an inhaled glucocorticoid and a LABA are a real possibility. Based on the information presented herein, we propose that a detailed molecular appreciation and pharmacodynamic understanding of the changes in gene expression induced by glucocorticoids and LABAs in relevant target and off-target cells and tissues will provide considerable new opportunities to...
Figure 2. An example of a conjugated inactive long-acting \( \beta_2 \)-adrenoceptor agonist glucocorticoid pro-drug

A phosphorylated form of salmeterol (blue) is shown conjugated to a derivative of the glucocorticoid, desisobutyryl-ciclesonide (pink). In vivo, the phosphate and ester bonds (in black) are cleaved (scissors) by alkaline phosphatase and esterases respectively to yield the active components in a 1:1 ratio. Adapted from [97].

Abbreviations: LABA, long-acting \( \beta_2 \)-adrenoceptor agonists.
improve the clinical efficacy of inhaled glucocorticoid/LABA combination therapy in asthma and COPD.

**Abbreviations**
cAMP, cyclic adenosine-3',5'-monophosphate; COPD, chronic obstructive pulmonary disease; LABA, long-acting β₂-adrenoceptor agonist; PDE, phosphodiesterase.

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