

EDITORIAL

Investigating the steroids and long-acting β_2 -agonists combination: why do we need more?

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Clinicians and scientists should work together, as the understanding of molecular mechanisms of diseases may help clinicians to improve their clinical practice. This is clearly exemplified by the studies on clinical and molecular interactions between long-acting inhaled β_2 -agonists (LABA) and glucocorticoids in the treatment of asthma and chronic obstructive pulmonary disease. Indeed, the possibility of obtaining a drug synergism combining in a single inhaler, LABA and inhaled glucocorticoids, in the regular long-term treatment of moderate-to-severe persistent asthma in adults has intrigued many clinicians and scientists in the last few years.

Inhaled β_2 -agonists are the most effective bronchodilators, and inhaled glucocorticoids the most effective anti-inflammatory drugs currently available for the treatment of asthma in adults. Since 1994 [1] many controlled clinical trials have conclusively demonstrated that the inhalation of a combination of low-dose glucocorticoids together with a LABA has at least the same efficacy as doubling the dose of the inhaled glucocorticoids in the long-term treatment of moderate-to-severe persistent asthma in adults [2].

Conventional wisdom had been that glucocorticosteroids and LABAs act through separate and distinct pathways. Studies on the efficacy of inhaled glucocorticoids in the treatment of asthma have demonstrated that they have a relatively flat dose/response curve with most of the benefit obtained at the lowest doses [3, 4]. The flatness of the dose/response means that the inflammation is suppressed by a relatively low dose of glucocorticoids. The addition of β_2 -agonists may exert some additional actions on the airways that is complementary to the effect of glucocorticoids.

In vitro and *in vivo* data demonstrates that β_2 -agonists might also influence the inflammatory process by affecting pro-inflammatory mechanisms other than relaxation of airway smooth muscle: in addition to their bronchodilator action, inhaled β_2 -agonists also inhibit mast cell mediator release, plasma exudation and may reduce sensory nerve activation [5].

Recently, several positive molecular interactions between inhaled β_2 -agonists and glucocorticoids have been identified. Glucocorticoids can upregulate the synthesis of several genes in human lung cells through interaction with specific DNA binding regions (glucocorticoid response elements) within the promoter region of glucocorticoid-responsive genes. Inhaled glucocorticoids increase the expression of β_2 -receptors by increasing gene transcription acting through glucocorticoid response elements [6], and, importantly, may regulate β_2 -adrenergic receptor function by restoring G-protein- β_2 -receptor coupling and inhibiting β_2 -receptor downregulation, thereby preventing its desensitisation [7]. Inhaled β_2 -agonists may

potentiate the molecular mechanism of action of glucocorticoids, with increased nuclear localisation of glucocorticoid receptors and additive or sometimes synergistic suppression of inflammatory mediator release [6]. Recently ROTH *et al.* [8] have elegantly documented a molecular mechanism where LABA potentiate the effects of glucocorticoids in airway smooth muscle. They found that the interaction of these two classes of drugs results in the synchronisation of the activity of the glucocorticoid receptor and C/EBP α which in turn achieves an optimum antiproliferative action *via* p21^{WAF1/Cip1} on smooth muscle cells.

In this issue of the *European Respiratory Journal (ERJ)* MYO *et al.* [9] add an important piece of knowledge on the molecular mechanisms of interaction, and possibly potentiation, between LABA and glucocorticoids. They report that salmeterol 10^{-7} M added after 24 h pre-treatment with fluticasone inhibited both interleukin (IL)-5 and eotaxin-induced eosinophil adhesion in a concentration dependent manner. The combination was synergic at fluticasone (FP) $\leq 10^{-9}$ M and additive at FP $\geq 10^{-8}$ M. This finding was clearly associated with a substantial blockade of phosphorylated cytosolic phospholipase A₂ (cPLA₂) translocation to the nuclear membrane, which is a step required for IL-5 stimulated eosinophil adhesion. The study is an extension of previous investigations on the role of cPLA₂ on eosinophil adherence and activation, which demonstrated the relevance of the cPLA₂ pathway in eosinophil adhesion *via* platelet activating factor [10, 11] and in leukotriene C4 (LTC4) production *via* arachidonate synthesis [12].

PLA₂ is the rate-limiting enzyme involved in the conversion of membrane phospholipids to arachidonic acid and lysophospholipids. Further catalysis of arachidonic acid initiates the biosynthesis of prostaglandins and leukotrienes, while lysophospholipids are converted directly into platelet-activating factor [13].

Eosinophils are one of the most abundant inflammatory cells recruited to the asthmatic airways. IL-5 plays an essential role in eosinophilic inflammation as demonstrated in IL-5 knockout mice where eosinophilia is markedly suppressed [14]. Conversely, data in humans are less conclusive [15, 16]. An initial step in the recruitment process is eosinophil adhesion by integrins to endothelial cell counterligands. The signal transduction pathways regulating eosinophil adhesion have not been entirely elucidated, however any inhibitory effect on this mechanisms, such as that described by MYO *et al.* [9] in this issue of the *ERJ*, would reduce the development of eosinophilic airway inflammation.

At variance with other studies on molecular mechanisms of interaction between inhaled glucocorticoids and LABA, the added value of the present study is that it documents a synergistic/additive effect of this combination on molecular pathways that are not typically considered steroid sensitive, *i.e.* the cascade of inflammatory mediators deriving from membrane phospholipid metabolism. Indeed, the relevance

of the study goes beyond finding a novel site of synergic interaction between the two classes of drugs, because it prospectively opens a new concept in this field, *i.e.* the search for positive interaction between LABA and inhaled glucocorticoids in pathways not usually considered as "classical" targets for glucocorticoids when used alone. This raises the possibility of exploring anti-inflammatory mechanisms of glucocorticoids currently not considered prominent. It would be interesting, for example, to investigate whether the addition of LABA would amplify the previously described suppressive effects of fluticasone on LTC₄ generation in eosinophils [12].

However, the study published in this issue of the *ERJ* is not entirely conclusive since it leaves some important mechanistic questions unanswered [9]. Indeed, neither the mechanisms by which salmeterol and/or fluticasone interfere with cPLA₂ nuclear translocation, nor the molecular mechanisms by which the inhibition of cPLA₂ nuclear translocation determines reduced eosinophil adhesion are investigated.

A surprising finding of the study by MYO *et al.* [9] that also deserves further investigations is the fact that FP at variance with other glucocorticoids (such as budesonide and dexamethasone) [17–19] does not reduce the expression of the integrin CD11b on the surface of activated eosinophils. Is this difference linked to a peculiar unknown mechanism of action of this drug?

The study by MYO *et al.* [9] also raises a more generic question: do we need more studies testing the effects, the efficacy and the molecular mechanisms of this drug combination? Certainly yes, if we consider the improvement in the scientific knowledge and the stimulating pharmacological questions that arise from studies like the present one. Probably no, if instead the aim is simply to increase the number of studies supporting the scientific rationale for the use of a combination of inhaled glucocorticoids and LABA in the treatment of asthma. In the last few years there has been a proliferation of *in vitro* and *in vivo* studies published in respiratory journals providing convincing evidence of the clinical efficacy of this association. How long should we look for "*a posteriori*" molecular support for what many controlled clinical trials have already demonstrated to be efficacious and safe? From the clinical point of view, such an experimental approach may be seen as an academic exercise of limited interest outside the "molecular biology" expert circles. Drug companies also should reflect on whether this is a true or false necessity, especially when their own data on file, and not published data, is quoted as reference for some critical methodological issues (for example to justify the concentrations of salmeterol utilised in this study).

As clinicians and scientists in respiratory medicine we need to look ahead to address pharmaceutical industry interests towards more intriguing targets. We cannot risk standing still on already acquired goals.

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