

Original article

Biochemical interaction between effects of beclomethasone dipropionate and salbutamol or formoterol in sputum cells from mild to moderate asthmatics

Background: Several *in vitro* studies demonstrate that corticosteroids and long-acting β_2 agonists may have a complementary and synergistic mode of action on the inflammatory processes in asthma.

Methods: Sputum was induced in 20 mild to moderate asthmatic patients and the induced sputum cells (ISC) were cultured with beclomethasone dipropionate (BDP) 10^{-7} M, salbutamol 10^{-8} M and formoterol 10^{-8} M either alone or in combination, BDP plus salbutamol and BDP plus formoterol, for 24 h. We measured the levels of growth macrophages-colony stimulating factor (GM-CSF), released on activation normal T cells expressed and activated (RANTES) and interleukin-8 (IL-8), in the supernatant of stimulated cells by ELISA. Furthermore, we assessed nuclear translocation of glucocorticoid receptor (GR) and the expression of β_2 receptor in ISC by immunofluorescence and RT-PCR, respectively.

Results: The release of GM-CSF, RANTES and IL-8 in ISC was significantly reduced by BDP plus salbutamol or formoterol as compared with either drug alone ($P < 0.0001$). β_2 receptor expression was increased after 30 min of incubation with BDP and continued to increase over a time period of 4 h ($P < 0.0001$). Furthermore after 30 min of incubation, nuclear translocation of GR was greater with BDP plus salbutamol or formoterol than with any of the drugs alone ($P < 0.0001$).

Conclusion: The present *ex vivo* study demonstrates a complementary mode of action between BDP and salbutamol or formoterol leading to an enhanced anti-inflammatory activity.

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The pathophysiology of bronchial asthma is characterized by the presence of a chronic inflammation where cell activation and cytokine networks play a critical role. Anti-inflammatory treatment with inhaled corticosteroids provides the mainstay of asthma therapy together with bronchodilation induced by β_2 agonists [short-acting β_2 agonists (SABA), long-acting β_2 agonists (LABA)]. It has been shown that SABA monotherapy can cause a rebound increase in bronchial responsiveness after cessation of therapy (1) and how early response to allergens could be increased by regular salbutamol administration (2). Several recent clinical studies,

however, particularly with LABA, have retired the claim concept that β_2 agonists are potentially harmful and may mask underlying inflammation (3–7). It is now demonstrated that when LABA are added to inhaled steroids in symptomatic patients despite regular treatment with inhaled corticosteroids, beneficial effects on symptoms, lung function and asthma exacerbations can be obtained (3–6). This suggests that the best treatment for moderate to severe persistent asthma is to combine an anti-inflammatory drug with a long-acting airway smooth muscle relaxant and that there is a beneficial interaction between LABA and corticosteroids on the inflammatory processes.

Recent evidences suggest potential anti-inflammatory actions of β_2 agonists, which may exert complementary or synergistic effects to corticosteroids (8, 9). It has been shown that, in fibroblasts and vascular smooth muscle cells, β_2 agonists may promote translocation of the glucocorticoid receptor (GR) into the cell nucleus (10)

Abbreviations: BDP, beclomethasone dipropionate; GM-CSF, growth macrophages-colony stimulating factor; GR, glucocorticoid receptor; IL-5, interleukin-5; IL-6, interleukin-6; IL-8, interleukin-8; ISC, induced sputum cells; LABA, long-acting β_2 agonist; RANTES, released on activation normal T cells expressed and activated; SABA, short-acting β_2 agonist.

suggesting an increase in the potency of corticosteroids when given concomitantly to β_2 agonists.

Although these results provided important evidence for understanding the mechanisms and the scientific rationale of the combination therapy, most of these findings have been obtained using airway cell lines stimulated with inflammatory stimuli. In order to explain how β_2 agonists and steroids in combination can inhibit the activation of airway cells and to provide an explanation for such additional effects, we assessed the ability of beclomethasone dipropionate (BDP) and salbutamol or formoterol to exert enhanced anti-inflammatory activities, correlated to biochemical interactions, by using induced sputum cells (ISC) isolated from mild to moderate asthmatics.

Materials and methods

Patients

The study included 20 patients suffering from mild to moderate asthma, diagnosed and classified according to the GINA (11). Smokers or ex-smokers, or who had experienced a severe asthma exacerbation (requiring hospitalization) during the month preceding the study, were excluded. Treatment with inhaled or oral corticosteroids was stopped at least 2 months before the start of the study, cromones were discontinued at least 2 weeks before the study, and theophylline was stopped 48 h prior to study initiation. Written informed consent was obtained from all patients prior to study entry and the study was approved by local Ethic Committees.

Sputum induction and processing

Sputum induction and processing were performed according to the method of Hargreave et al. (12) with minor modification (13).

Incubation of induced sputum cells with BDP, salbutamol and formoterol

Induced sputum cells were resuspended at a concentration of 1×10^6 /ml in RPMI 1640 + 10% heat-inactivated (56°C, 30 min) FCS + 1% penicillin-streptomycin solution + 1 mmol/l L-glutamine. ISC were cultured in the absence or presence of BDP (10^{-7} M) and BDP (10^{-7} M) plus salbutamol (10^{-8} M) or plus formoterol (10^{-8} M) for 30 min, 4 h and 24 h at 37°C in a humidified 5% CO₂. The concentration range of the drugs was previously shown (10, 14). At the end of incubation the cell viability was > 85%, as assessed by trypan blue dye exclusion. The supernatants and cells of stimulated ISC cultures were harvested and stored at -80°C until analysis.

Measurement of GM-CSF, RANTES and IL-8

Determinations of the absolute values of growth macrophages-colony stimulating factor (GM-CSF), released on activation normal T cells expressed and activated (RANTES) and interleukin-8 (IL-8) in the supernatants of stimulated ISC in the absence or presence of

BDP (10^{-7} M) and BDP (10^{-7} M) plus salbutamol (10^{-8} M) or plus formoterol (10^{-8} M) for 24 h at 37°C in a humidified 5% CO₂, were assessed using commercially available specific enzyme immunoassay kits (ELISA) (R&D System, Minneapolis, MN). The detection limit of GM-CSF, RANTES and IL-8 kits were 18, 3 and 10 pg/ml, respectively.

RT-PCR of β_2 receptor

Extraction of total RNA from stimulated ISC in the absence or presence of BDP (10^{-7} M) for 30 min and 4 h at 37°C in a humidified 5% CO₂, was performed as previously described using TRIzol reagent (Gibco BRL, Milan, Italy) (15).

The sequences of oligonucleotide primers used in PCR were:

β -actin: sense 5' ATTGGCAATGAGCGGTTCCG 3', antisense 5' CCGCCGATCCACACGGAGTA 3'

β_2 receptor: sense 5' TCAGCAGCAAAGGGACGAG 3', antisense 5' GCGGCCCTCAGATTTGTCAA 3'

The predicted sizes of amplification products were 281 bp in β -actin, 647 bp in β_2 receptor.

Thirty cycles were used, with a denaturing step at 94°C for 45 s, followed by the specific primer annealing temperature 50°C for 45 s and an extension step at 72°C for 1 min. β -Actin was used as a positive control for the PCR reaction and a no template cDNA control as the negative control. PCR products were analyzed by electrophoresis on ethidium-bromide stained 1.8% agarose gels. The gels were visualized under UV light exposure.

Densitometric analysis of PCR products of β_2 receptor

The PCR products of β_2 receptor were quantified by densitometric scanning by using a NIH Image 1.61 program and normalized against β -actin. Pictures of the gel were taken by using an EPSON GT-6000 scanner and the image was imported to an NIH Image analysis program to determine band density using surface under the curve.

Immunofluorescence

Nuclear translocation of GR was assessed by immunofluorescence technique in ISC stimulated in the absence or presence of BDP (10^{-7} M) and BDP (10^{-7} M) plus salbutamol (10^{-8} M) or plus formoterol (10^{-8} M) for 30 min at 37°C in a humidified 5% CO₂. Cytospins for immunofluorescence were prepared as previously described (16). Thawed cytopins of ISC were treated with 2% gelatin to block nonspecific IgG binding and were then incubated with polyclonal antibody direct against the α isoform anti-GR α (polyclonal) (Affinity Bioreagents; Golden, CO, USA), anti-CD18 mAb (CLONE MHM23; Dakopatts, Glostrup, Denmark). Primary antibody binding was detected with FITC-conjugated anti-mouse IgG Ab (Sigma, Co., Milan, Italy) or TRITC-conjugated anti-rabbit IgG Ab (Sigma). GR translocation was detected by colocalization with bisbenzamide (HOECHST) and cell population was quantified by assessing 400 immunopositive cells for each experimental condition. Results were expressed as percentage of positive macrophages with nuclear translocation.

Statistical analysis

Results were expressed as medians and 25–75th percentiles. Statistical analysis of the data was performed with ANOVA test with Bonferroni–Dunn correction.

Results

Demographic and cellular characteristics of patients

The demographic characteristics of patients are reported in Table 1. Cell analysis of sputum cells is represented in Table 2.

Release of GM-CSF, RANTES and IL-8 by cultured ISC

The GM-CSF release by cultured ISC not treated with drug was 720 (695–789) pg/ml and decreased after BDP treatment to 378 pg/ml (332–445) ($P < 0.0001$). The release of GM-CSF was significantly reduced by BDP plus salbutamol or plus formoterol [BDP plus salbutamol: 300 pg/ml (225–310); BDP plus formoterol: 222.5 pg/ml (170–250)] as compared with either drug alone [salbutamol: 701 pg/ml (684.5–760); formoterol 700 pg/ml (668–743)] ($P < 0.0001$) (Fig. 1).

At baseline RANTES released by cultured ISC was 557 (475–575) pg/ml and decreased after BDP treatment to 294 pg/ml (240–300) ($P < 0.0001$). The release of RANTES was significantly reduced by BDP plus salbutamol or formoterol [BDP plus salbutamol: 190 pg/ml (180–207); BDP plus formoterol: 150 pg/ml (100–175)] as compared with either drug alone [salbutamol 535 pg/ml (490–560); formoterol 520 pg/ml (480–540)] ($P < 0.0001$) (Fig. 2).

The baseline level of IL-8 by cultured ISC was 4457 (4475–4575) pg/ml and decreased after BDP treatment to 3294 pg/ml (3234–3303) ($P < 0.0001$). The release of IL-8 was significantly reduced by BDP plus salbutamol or

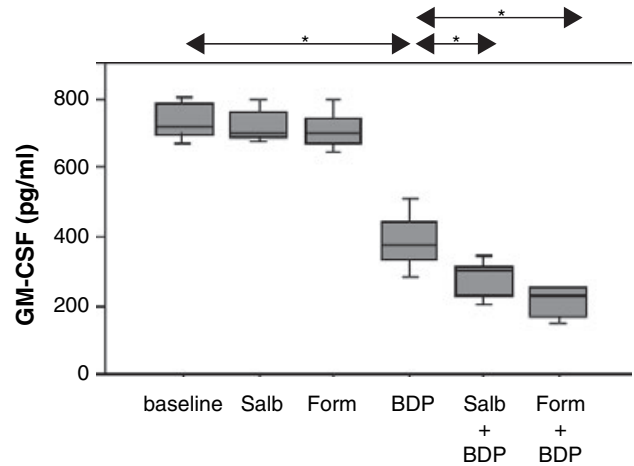


Figure 1. Effects of BDP (10^{-7} M), BDP (10^{-7} M) plus salbutamol (10^{-8} M) and BDP plus formoterol (10^{-8} M) on the release of GM-CSF by ISC. The cells were incubated for 24 h with the drugs. The release of GM-CSF was significantly reduced by BDP plus formoterol or salbutamol as compared with either drug alone ($*P < 0.0001$). Statistical analysis was performed by ANOVA with Bonferroni correction.

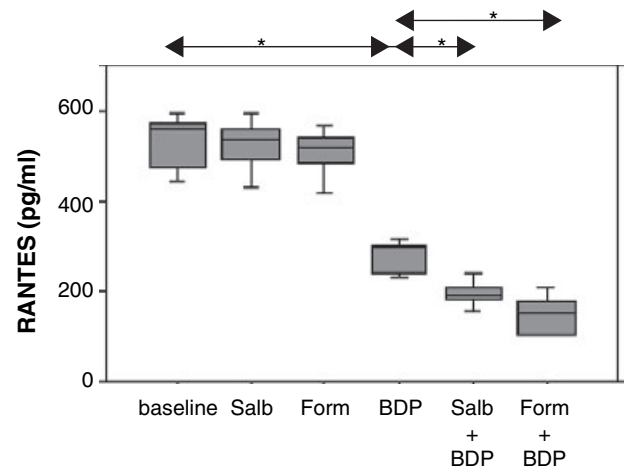


Figure 2. Effects of BDP (10^{-7} M), BDP (10^{-7} M) plus salbutamol (10^{-8} M) and BDP plus formoterol (10^{-8} M) on the release of RANTES by ISC. The cells were incubated for 24 h with the drugs. The release of RANTES was significantly reduced by BDP plus formoterol or salbutamol as compared with either drug alone ($*P < 0.0001$). Statistical analysis was performed by ANOVA with Bonferroni correction.

Table 1. Demographic characteristic of patients

	Asthma
Number	12
Male/female	7/12
Age (years)*	34 (28.5–46)
Smokers	0
FEV ₁ (%)	94 (76.2–101.5)

*Data are expressed as median with 25–75th percentiles.

Table 2. Cell analysis of sputum samples

Viability	72.9 (68.9–78.3)
Volume (ml)	1.1 (0.9–2.1)
Squamous cells (%)	6.3 (2.1.3–10.6)
Cell counts (million cells/ml)	1 (0.9–152)
Differential cell counts (%)	
Macrophages	36.9 (27.4–52.8)
Neutrophils	41.1 (24.6–52.6)
Lymphocytes	0.5 (0.2–0.9)
Eosinophils	13.7 (6.7–24.5)
Epithelial cells	0

formoterol [BDP plus salbutamol: 2990 pg/ml (2839–3000); BDP plus formoterol: 2150 pg/ml (2102–2190)] as compared with either drug alone [salbutamol: 4535 pg/ml (4432–4575); formoterol 4349 pg/ml (4234–4404)] ($P < 0.0001$) (Fig. 3).

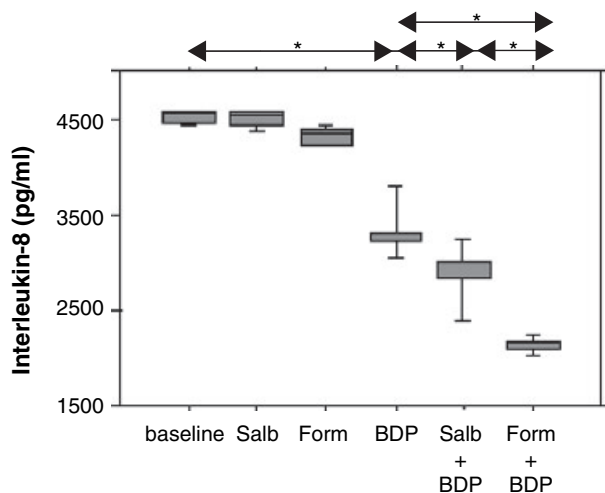


Figure 3. Effects of BDP (10^{-7} M), BDP (10^{-7} M) plus salbutamol (10^{-8} M) and BDP plus formoterol (10^{-8} M) on the release of IL-8 by ISC. The cells were incubated for 24 h with the drugs. Statistical analysis was performed by ANOVA with Bonferroni correction. The release of IL-8 was significantly reduced by BDP plus formoterol or salbutamol as compared with either drug alone. Statistical analysis was performed by ANOVA with Bonferroni correction (* $P < 0.0001$).

β_2 -adrenergic receptor in ISC treated with BDP

RT-PCR of β_2 receptor was performed on ISC stimulated in the presence or absence of BDP (10^{-7} M) at 30 min and at 4 h. The results show that the transcription for β_2 receptor was significantly increased in ISC after 30 min of incubation with BDP (10^{-7} M) and was further increased after 4 h ($P < 0.0001$) (Fig. 4).

Selective translocation of GR

The translocation of GR was significantly increased in stimulated ISC by BDP or by BDP plus salbutamol or formoterol as compared with either drug alone ($P < 0.0001$) (Fig. 5). The GR nuclear activation and translocation was specifically present in sputum macrophages and eosinophils. The percentage of macrophages showing nuclear translocation of GR α was significantly increased by BDP [14.9 (12.7–22)] or by BDP plus salbutamol or plus formoterol [BDP plus salbutamol: 21.5% (17.7–27.5); BDP plus formoterol: 27% (20.4–30)] as compared with baseline [3.1% (3–6)] or either drug alone [salbutamol: 3.8% (3–5.5); formoterol 4% (3.2–4.7)] ($P < 0.0001$, respectively). The percentage of eosinophils showing nuclear translocation of GR α was significantly increased by BDP [2.8% (2.2–3.4)] or by BDP plus salbutamol or plus formoterol [BDP plus salbutamol: 4.4% (3.4–6); BDP plus formoterol: 4.8% (4–7.5)] as compared with baseline [0% (0–0.1)] or to either drug alone (salbutamol: 0% (0–0.1); formoterol 0% (0–0.1)] ($P < 0.0001$, respectively). No differences in the nuclear translocation of

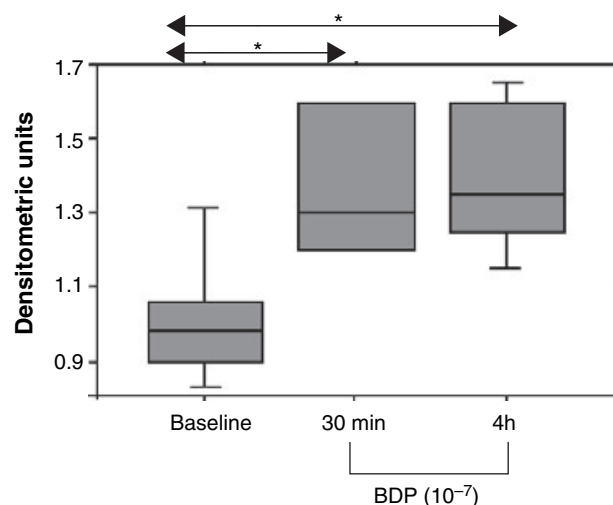


Figure 4. Effects of BDP (10^{-7} M) on the transcription of the β_2 receptor mRNA in the ISC. incubated with BDP for 30 min and 4 h. Equal amount of total RNA (2 ng) obtained from SC were reverse transcribed into cDNA. The densitometric units in the histogram reflect the quantitative levels of β_2 receptor mRNA normalized to the constant levels of β -actin. Statistical analysis was performed by ANOVA with Bonferroni correction (* $P < 0.0001$).

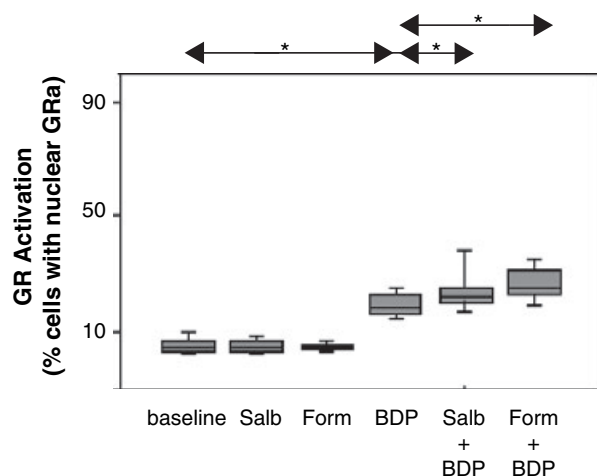


Figure 5. Effects of BDP (10^{-7} M) plus salbutamol (10^{-8} M) and BDP plus formoterol (10^{-8} M) on the nuclear translocation of GR in the ISC stimulated for 30 min. Quantitative analysis of GR nuclear translocation was performed by calculating the percentage of total ISC in which GR colocalized with bis-benzimide. Statistical analysis was performed by ANOVA with Bonferroni correction (* $P < 0.0001$).

GR α for neutrophils, lymphocytes and epithelial cells were detected (Table 3).

Discussion

Asthma is a complex condition deriving from the interaction of several environmental factors acting on a

background of genetic predisposition. This disease is characterized by airway inflammation, reversible airway obstruction and airway hyper-responsiveness to different stimuli. Asthma is a progressive disease that is treated with a range of therapies according to its severity (17).

Mild to moderate asthma is characterized by a high clinical heterogeneity and many of these patients may experience severe exacerbations as well as daytime and nocturnal symptoms. Patients with mild to moderate asthma are treated mainly in the primary care setting, often without inhaled corticosteroids and consequently the use of β_2 agonists alone may mask the presence of an underlying inflammation. Several clinical studies have shown that adding long-acting β_2 agonists to the treatment of symptomatic patients despite regular treatment with inhaled corticosteroids produces beneficial effects on symptoms, lung function and asthma exacerbations (3–7). Bouros treated asthmatic patients not controlled with regular use of inhaled steroids with the combination of BDP and formoterol or a double dose of BDP without adding regular bronchodilator. This study showed that BDP and formoterol combined therapy is more effective than doubling the dose of BDP without adding formoterol (18). These findings confirm the presence of a beneficial molecular anti-inflammatory interaction between β_2 agonists and corticosteroids.

Airway inflammatory cells may considerably contribute to the establishment and/or to the maintenance of airway inflammation by the release of cytokines such as GM-CSF, RANTES, IL-8, IL-5 and IL-6 (19). Functional β_2 receptors have been detected in mast cells, monocytes/macrophages, neutrophils, eosinophils and T lymphocytes (20) and both SABA and LABA inhibit inflammatory mediator release from these cells (14). Moreover, β_2 agonists and corticosteroids, when used in combination, may have additive and or synergic effects on both inflammatory cells (20) and epithelial cells (14).

Interestingly, in the present study GM-CSF, RANTES and IL-8 were used as markers for ISC activation and response to glucocorticoids treatment. We found that ISC isolated from mild to moderate asthmatic patients spontaneously released high amounts of GM-CSF, RANTES and IL-8 and that the release of these cytokines was

down-regulated after BDP treatment. In addition, such inhibition was significantly higher in the presence of BDP in combination with salbutamol or formoterol, demonstrating that there is functional synergy between BDP and the two tested β_2 agonists in terms of anti-inflammatory effect in patients with mild-moderate asthma. It is of note that differences between BDP plus salbutamol and BDP plus formoterol in terms of synergistic down-regulatory effect on the release of the tested cytokines were found only for IL-8 release. The combination between BDP and formoterol was able to further reduce the IL-8 release by ISC when compared with the combination between BDP and salbutamol. How both salbutamol and formoterol interact with BDP to reduce IL-8 release is unknown and, consequently, a definitive explanation of this phenomenon is not presently available. In the same set of experiments, we show that, although in the absence of a statistical difference, the presence of salmeterol alone was able to smoothly down-regulate the release of IL-8 when compared with the presence of medium or salbutamol alone. Tobler et al. demonstrated that steroids are able to down-regulate the release of IL-8 by epithelial cells via the reduction of the half life of IL-8 messenger ribonucleic acid (mRNA) (21). It is conceivable that the synergistic effect displayed by salmeterol and BDP for IL-8 release is exerted via this described mechanism which has not been described for GM-CSF or RANTES.

Our findings are consistent with previous observations demonstrating a synergistic effect between inhaled corticosteroids and LABA on asthma control (22) as well as on the release of some proinflammatory mediators by immunocompetent peripheral blood cells (20) or bronchial epithelial cells (14). To our knowledge this is the first report demonstrating such effect in proinflammatory cells freshly isolated from induced sputum of asthmatic patients.

Corticosteroids can modulate β_2 receptors by the activating β_2 receptor gene. Accordingly, it has been shown that intranasal administration of BDP increases the density of β_2 receptor on the nasal mucosa and that corticosteroids can prevent or reverse β_2 receptor (23) down-regulation. In the present study we have also demonstrated that GR-ligand activation may regulate β_2

Table 3. Translocation of GR in ISC

Positive cells (%)	Baseline	Salb	Form	BDP	Salb plus BDP	Form plus BDP	P values		
							BDP/Salb + BDP	BDP/Form + BDP	Salb + BDP/Form + BDP
Macrophages	3.01 (3–6)	3.08 (3–5.5)	4 (3.2–4.7)	14.09 (12.7–22)	21.05 (17.7–27.5)	27 (20.4–30)	<0.0001	<0.0001	<0.0001
Neutrophils	0.01 (0–0.4)	0 (0–0.3)	0 (0–0.4)	0.05 (0.2–1)	0.05 (0.2–1)	0.05 (0.2–1)	NS	NS	NS
Eosinophils	0 (0–0.1)	0 (0–0.1)	0 (0–0.1)	2.08 (2.2–3.4)	4.04 (3.4–6)	4.08 (4–7.5)	<0.0001	<0.0001	<0.0001
Lymphocytes	0.01 (0–0.3)	0.01 (0–0.3)	0.01 (0–0.2)	0 (0–0.1)	0 (0–0.1)	0 (0–0.5)	NS	NS	NS
Epithelial cells	0 (0–0.1)	0 (0–0.1)	0 (0–0.1)	0 (0–0.1)	0 (0–0.1)	0.05 (0–0.4)	NS	NS	NS
Total cells	4.3 (3.1–6.6)	4.3 (3.1–6.6)	4.6 (3.7–5.1)	19.7 (16.5–24.1)	28.3 (22.5–32.9)	31.2 (26.7–37)	<0.0001	<0.0001	<0.0001

Data are expressed as median with 25–75th percentiles. Salb, salbutamol; Form, formoterol; BDP, beclomethasone dipropionate.

receptor function, as shown by the increased expression of these receptors in ISC from asthmatic patients after exposure to BDP. Indeed, we found an overexpression of the mRNA for β_2 receptors due to the incubation with BDP that initiated after 30 min and continued for a period of 4 h. This mechanism is very rapid and it may be explained by a cessation of mRNA degradation. This effect could be due to BDP-induced interactions with RNAi (interfering RNAs) (21). To better understand this regulatory issue, future studies using the addition of traditional transcription inhibitors would be performed.

The increased amount of β_2 agonists mRNA, demonstrates that the synergistic effect of BDP and β_2 agonists is also due to the ability of steroids to increase the expression of β_2 receptor. This increase in the expression of β_2 receptors has already been demonstrated (24) but it has never been observed in proinflammatory cells freshly isolated from induced sputum of asthmatic patients. These findings strongly support the hypothesis of an increased anti-inflammatory role of steroids when used in combination with β_2 agonists.

The mechanism by which β_2 agonists exert their anti-inflammatory role is not completely understood. One possibility might be related to the direct interaction between the transcription factors and the GR. It is known that the activation of protein kinases A and C stimulates GR activities by phosphorylation of specific sites at the N-terminus side of the GR (10, 25) priming it for subsequent steroid binding (20). It has been suggested that the biochemical modulation of GR is achieved by phosphorylation (activation) and dephosphorylation (inactivation) at seven different phosphorylation sites (26–28) GR rapidly translocates from the cytosol into the nucleus resulting in a modulated transcription of several genes, such as cytokines and other mediators involved in inflammatory process. Interestingly, studies on fibroblasts and vascular smooth muscle cells showed that β_2 agonists promote GR activation and nuclear translocation (10, 25), suggesting that this may increase the potency of corticosteroids given concomitantly β_2 agonists. In this study we found, for the first time, that the combination of BDP and salbutamol or formoterol activates GR translocation in a synergistic mode of action as compared with BPD alone in cells isolated from induced sputum of mild-moderate asthmatic patients and specifically in macrophages and eosinophils. This provides a strong

scientific rationale for the combined use of BDP and the two tested short- and long-acting β_2 agonists. The inhibition by formoterol of cytokine release by GR activation was not observed by Korn et al. (14) in a study using an epithelial cell line. One explanation for this contrasting observation could be that β_2 agonist activity may differ depending on the cell type target. Another explanation could be the fact that because our cells were freshly isolated from induced sputum of asthmatic patients, they might have a different susceptibility to the effect of different drugs. Further studies are needed to better understand these mechanisms.

Whereas rational and clinical consequences of combining inhaled corticosteroids with LABA are well known, an interesting finding of our study is that the SABA salbutamol seems to produce the same beneficial *in vitro* effects as a LABA when added to BDP in terms of biochemical interaction leading to enhanced anti-inflammatory activity and expression of the β_2 receptor.

These findings may explain previous data showing beneficial effects of BDP and salbutamol, in preventing allergen-induced bronchoconstriction measured by pulmonary function tests (29, 30). Moreover, these concepts may support the use of salbutamol combined with BDP instead of salbutamol alone in symptomatic relief of asthmatic patients, particularly those who are not regularly treated with inhaled corticosteroids [i.e. intermittent asthma or under-treated mild persistent asthma (31, 32)]. A well-designed, randomized controlled clinical trial is needed to verify and support this hypothesis. Furthermore, future studies should be aimed to identify more sensitive sputum markers able to assess the anti-inflammatory response to combined therapy between corticosteroids and SABA or LABA.

In conclusion, the present *ex vivo* study on ISC demonstrates a synergistic mode of action of both SABA and LABA combined with inhaled corticosteroids on some anti-inflammatory effects. The specific use of both SABA and LABA in this study and their synergism with BDP in down-regulating some anti-inflammatory effects on freshly isolated cells from asthmatics airways, provides additional evidences supporting the strong value of a combined therapy in asthma. Further studies aimed to increase the knowledge on specific biochemical mechanisms of interaction between corticosteroids and β_2 agonists are needed.

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