Effect of Ambroxol and Beclomethasone on Lipopolysaccharide-Induced Nitrosative Stress in Bronchial Epithelial Cells

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Introduction

Oxidative stress is an underlying pathogenetic mechanism of many chronic inflammatory disorders. In respiratory diseases such as chronic obstructive pulmonary disease (COPD), oxidative stress and high levels of pro-

Key Words
Ambroxol · Beclomethasone · In vitro study · Oxidative stress · Nitrosative stress · Chronic obstructive pulmonary disease

Abstract

Background: Nitrosative stress is involved in different airway diseases. Lipopolysaccharide (LPS) induces neutrophil-related cytokine release and nitrosative stress in human bronchial epithelial (BEAS-2B) cells alone or with human polymorphonuclear neutrophils (PMNs). Ambroxol protects against oxidative stress, and beclomethasone dipropionate is an anti-inflammatory drug. Objectives: We evaluated the ability of ambroxol and/or beclomethasone dipropionate to inhibit LPS-induced expression/release of RANTES, IL-8, inducible NO synthase (iNOS), myeloperoxidase (MPO) and 3-nitrotyrosine (3-NT: nitrosative stress biomarker) in BEAS-2B ± PMNs stimulated with LPS (1 μg/ml). Methods: The effect of ambroxol and/or beclomethasone dipropionate on IL-8, RANTES and iNOS levels was assessed by Western blot analysis; IL-8, MPO and 3-NT levels were measured by ELISA. Cell viability was assessed by the trypan blue exclusion test. Results: In BEAS-2B alone, LPS (at 12 h) increased RANTES/iNOS expression and IL-8 levels (p < 0.001). Ambroxol suppressed LPS-induced RANTES expression and IL-8 release (p < 0.001), whilst inhibiting iNOS expression (p < 0.05). Beclomethasone dipropionate had no effect on RANTES but halved iNOS expression and IL-8 release. Coculture of BEAS-2B with PMNs stimulated IL-8, MPO and 3-NT production (p < 0.001), potentiated by LPS (p < 0.001). Ambroxol and beclomethasone dipropionate inhibited LPS-stimulated IL-8, MPO and 3-NT release (p < 0.05). Ambroxol/beclomethasone dipropionate combination potentiated the inhibition of IL-8 and 3-NT production in BEAS-2B with PMNs (p < 0.05 and p < 0.01, respectively). Ambroxol and/or beclomethasone dipropionate inhibited nitrosative stress and the release of neutrophilic inflammatory products in vitro. Conclusion: The additive effect of ambroxol and beclomethasone dipropionate on IL-8 and 3-NT inhibition suggests new therapeutic options in the treatment of neutrophil-related respiratory diseases such as chronic obstructive pulmonary disease and respiratory infections.
inflammatory endogenous nitric oxide (NO), which plays a key role in the physiological regulation of airway function, produce strong oxidizing reactive nitrogen species [1, 2], which reinforce inflammation, damage DNA and inhibit mitochondrial respiration, causing protein dysfunction and cell damage. This, together with oxidative stress, is nitrosative stress [1].

A study investigating nitrosative stress markers, including 3-nitrotyrosine (3-NT), inducible NO synthase (iNOS), endothelial NO synthase, myeloperoxidase (MPO) and xanthine oxidase in bronchial biopsies from patients with stable COPD, showed that MPO+ and 3-NT+ cells and neutrophils are markedly increased in severe COPD, indicating that MPO and neutrophilic inflammation are key features of nitrosative stress [3]. Bradykinin and lipopolysaccharide (LPS) stimulate neutrophil chemo-attractant interleukin (IL)-8 release and production of 3-NT and MPO (a marker of neutrophil activation) by human bronchial epithelial (BEAS-2B) cells cultured alone or with human polymorphonuclear neutrophils (PMNs) [4]. Furthermore, epithelial cells release IL-8 and 3-NT after stimulation by viruses [5], nitrogen dioxide [6] and acute lung injury [7].

Ambroxol hydrochloride, a mucolytic anti-inflammatory agent used to treat respiratory diseases associated with increased mucus production, has a wide range of properties including inhibition of oxidative and nitrosative stress [8–12]. Ambroxol has been shown to prevent generation of reactive oxygen species by broncho-alveolar lavage cells from COPD patients [13] suggesting that it may be useful in the management of COPD [14, 15]. Moreover, ambroxol’s antioxidant properties may be of clinical benefit in virus-induced infections. Indeed, several viruses, including influenza and respiratory syncytial virus, promote an intracellular redox state imbalance toward pro-oxidant conditions, thereby contributing to virus-induced damage to the host tissues [16]. Ambroxol improved the survival rate of mice after infection with a lethal dose of influenza virus [17] and can help prevent common acute upper respiratory diseases such as the common cold and influenza [18]. Beclometasone dipropionate is a glucocorticoid used in maintenance therapy for persistent asthma and in the prevention of asthma and COPD exacerbations [19–21]. These drugs may inhibit nitrosative stress and neutrophilic activity [22] and this could be demonstrated using the BEAS-2B bronchial epithelial cell model which already showed a sensitivity to glucocorticoids in terms of vascular cell adhesion molecule-1 expression inhibition as glucocorticoid anti-inflammatory effect [23].

The aim of this study was to evaluate the effect of ambroxol hydrochloride and/or beclometasone dipropionate on the LPS-induced release of neutrophil-related cytokines such as IL-8 and ‘regulated on activation, normal T lymphocyte expressed and secreted’ (RANTES/CLL5), expression of iNOS and MPO, and production of 3-NT in BEAS-2B cells alone or in coculture with human PMNs.

**Materials and Methods**

**Bronchial Epithelial Cell Culture**

Immortalized, non-tumorigenic human bronchial epithelial cells BEAS-2B (CRL-9609; American Type Culture Collection, Manassas, Va., USA) were cultured in a serum-free medium composed of 50% LHC9 medium (Gibco, Grand Island, N.Y., USA) and 50% RPMI-1640 medium (Euroclone Ltd., Paignton, UK) supplemented with 1× non-essential amino acids (Gibco), L-glutamine (2 mM), penicillin (100 U/ml)/streptomycin (100 U/ml; Euroclone Ltd.), and incubated at 37 °C in 100% humidity and 5% CO2 [4].

Additionally, in some experiments human PMNs were cocultured with BEAS-2B cells in 6-well plates (Falcon, BD Biosciences) equipped with porous cell culture membrane inserts with a pore diameter of 1 μm (Falcon, BD Biosciences) as previously described [24]. The study conformed to the local ethic criteria concerning the management of biological samples (PMNs from venous blood of healthy subjects).

PMNs were isolated from peripheral blood by standard dextran sedimentation followed by Histopaque® (Sigma-Aldrich, St. Louis, Mo., USA) gradient centrifugation. The PMNs were resuspended in Hepes-buffered saline solution (Clonetics, Basel, Switzerland) with a final cell suspension purity average of 98%. PMN viability (assessed by the trypan blue exclusion test) was always >95%.

**Experimental Study Design**

Evaluation of neutrophil-related mediators: when 80% confluent, BEAS-2B cells were incubated for up to 12 h with 1 μg/ml LPS from *Pseudomonas aeruginosa* serotype 10 (Sigma-Aldrich) in order to perform either a time course (0 min, 30 min, 1, 2, 4, 8 and 12 h) or a dose-response curve (from 10⁻⁹ to 10⁻⁵ M) to assess the effect of ambroxol hydrochloride (Chiesi Farmaceutici, Parma, Italy) or beclometasone dipropionate (Chiesi Farmaceutici) on RANTES and iNOS expression and on IL-8 release.

Evaluation of neutrophil-related markers of nitrosative stress: assessment of IL-8, MPO and 3-NT production by BEAS-2B cells either alone or cocultured with human PMNs at different time points (1, 2, 4 and 8 h) with and without LPS was performed. The effect of ambroxol and/or beclometasone on LPS-induced expression of these markers was also examined in BEAS-2B cells cocultured with human PMNs.

Specifically, the 9 groups were:

1. BEAS-2B cells alone;
2. BEAS-2B cells treated with LPS (1 μg/ml);
3. BEAS-2B cells treated with LPS (1 μg/ml) and ambroxol (from 10⁻⁹ to 10⁻⁵ M);

4. BEAS-2B cells alone;
5. BEAS-2B cells treated with LPS (1 μg/ml);
6. BEAS-2B cells treated with LPS (1 μg/ml) and beclomethasone (from 10⁻⁹ to 10⁻⁵ M);
7. BEAS-2B cells treated with LPS (1 μg/ml) and ambroxol (from 10⁻⁹ to 10⁻⁵ M);
8. BEAS-2B cells treated with LPS (1 μg/ml) and beclomethasone (from 10⁻⁹ to 10⁻⁵ M);
9. BEAS-2B cells treated with LPS (1 μg/ml) and ambroxol (from 10⁻⁹ to 10⁻⁵ M) and beclomethasone (from 10⁻⁹ to 10⁻⁵ M).
(4) BEAS-2B cells treated with LPS (1 μg/ml) and beclomethasone (from 10\(^{-9}\) to 10\(^{-6}\) M);
(5) BEAS-2B cells cocultured with PMNs;
(6) BEAS-2B cells cocultured with PMNs and treated with LPS (1 μg/ml);
(7) BEAS-2B cells cocultured with PMNs treated with LPS (1 μg/ml) and ambroxol (from 10\(^{-9}\) to 10\(^{-5}\) M);
(8) BEAS-2B cells cocultured with PMNs treated with LPS (1 μg/ml) and beclomethasone (from 10\(^{-9}\) to 10\(^{-5}\) M);
(9) BEAS-2B cells cocultured with PMNs treated with LPS (1 μg/ml) and the combination of ambroxol and beclomethasone (from 10\(^{-9}\) to 10\(^{-6}\) M).

In each experimental setting including ambroxol and/or beclomethasone, the concentration of the drug able to reach the maximal inhibition, according to the dose-response curves, was used and the drugs were added in the last 4 h of incubation. In each experimental setting including PMNs, no data were reported after 8 h of incubation with PMNs because of a significant neutrophil migration across the porous cell culture membranes after this time point.

Finally, in BEAS-2B cells, the effect of ambroxol (10\(^{-6}\) M) on p38 MAPK phosphorylation after treatment with LPS (1 μg/ml) was assessed.

**Western Blot Analysis**

To evaluate RANTES and iNOS expression, BEAS-2B cells were cultured alone or treated with LPS in the absence or presence of ambroxol or beclomethasone as previously described.

Activation of p38 MAPK was expressed by the increase in the phosphorylated p38 (p-p38)/p38 ratio. To evaluate p-p38 and p38, BEAS-2B cells were incubated for 12 h alone, with LPS, and with LPS plus the addition of ambroxol (10\(^{-6}\) M).

After incubation, cells were washed in PBS and lysed in 1 ml/dish of lysis buffer (20 mM Tris HCl at pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1% sodium dodecyl sulphate, 1 mM ethylene glycol tetra-acetic acid, 1 mM ethylenediamine tetra-acetic acid, 1 mM Na orthovanadate and 1× protease inhibitor cocktail; Sigma-Aldrich).

Total cell lysates were incubated in ice for 20 min, centrifuged at 9,280 g for 10 min at 4 °C, and their protein concentration was quantified by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, Calif., USA). Protein extracts (50 μg/well) were separated on 4–15% non-reducing sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (Bio-Rad).

Blots were incubated with goat anti-RANTES antibody (1:1,000, R & D Systems, Abingdon, UK), mouse anti-iNOS2 (iNOS) antibody (1:200, Santa Cruz Biotechnology), and rabbit antibodies to total p38 or p-p38 (1:1,000, New England Biolabs, Beverly, Mass., USA) and visualized using the enhanced chemiluminescence system (Amersham Biosciences, Little Chalfont, UK).

Blots were then stripped using the Restore ltd Western Blot Stripping Buffer (Thermo Scientific, Waltham, Mass., USA) and reprobed with anti-β-actin antibody (clone C4; 1:1,000, Boehringer Mannheim Inc., Mannheim, Germany). The relevant band intensities were analysed using the NIH Image J 1.38x program.

**Results**

**Time Course of LPS-Induced RANTES and iNOS Expression and IL-8 Release in BEAS-2B Cells**

RANTES and iNOS proteins were constitutively expressed and IL-8 was tonically released by BEAS-2B cells (fig. 1a–c). The expression of RANTES and iNOS, and IL-8 release did not change over 12 h (data not shown). LPS induced a time-dependent increase in RANTES and iNOS expression (0 min, 30 min, 1, 2, 4, 8 and 12 h) as shown in figure 1a and c. In addition, LPS enhanced IL-8 concentration in a time-dependent manner (fig. 1b).

**Dose-Response Curves for Ambroxol and Beclomethasone in BEAS-2B Cells**

Dose-response curves from 10\(^{-9}\) to 10\(^{-5}\) M of ambroxol and beclomethasone at 12 h were performed (data not shown). The drug concentrations that achieved the maximal inhibition in each experimental setting (stated in each section below) were used to assess the drug effect on LPS-induced cytokine release and expression of nitrosative stress markers.

**Effect of Ambroxol and Beclomethasone on RANTES and iNOS Expression and IL-8 Release in BEAS-2B Cells**

The drug concentrations that achieved the maximal inhibition in this setting were 10\(^{-7}\) M for ambroxol and...
10^{-6} M for beclomethasone. Ambroxol 10^{-7} M suppressed the LPS-induced expression of RANTES and IL-8, whereas beclomethasone 10^{-6} M had no effect on LPS-induced RANTES expression, but halved IL-8 release (fig. 2a, b). The effect of ambroxol (97% of inhibition) on IL-8 release was significantly greater than that of beclomethasone (53% of inhibition; p < 0.05; fig. 2b). On the other hand, beclomethasone and ambroxol both inhibited LPS-induced iNOS expression by 52 and 30%, respectively (fig. 2c).

**LPS-Induced IL-8, MPO and 3-NT Release in BEAS-2B Cells Cocultured with Neutrophils**

The presence of neutrophils in coculture with BEAS-2B cells, compared to BEAS-2B cells alone, increased MPO and 3-NT release starting from the first hour of incubation, whilst for IL-8 release the effect of neutrophils was evident after 2 h of incubation (fig. 3). The maximal effect of the coincubation of neutrophils with BEAS-2B cells was reached after 8 h for each marker (fig. 3).

LPS induced a further enhancement of IL-8 and MPO release by BEAS-2B co-incubated with neutrophils starting at 2 and 1 h, respectively, reaching the maximal effect at 8 h (fig. 3a, b). LPS produced a higher increase in 3-NT release by BEAS-2B co-incubated with neutrophils starting at 4 h and reaching the maximal effect at 8 h (fig. 3c).

**Ambroxol and Beclomethasone Effects on IL-8, MPO and 3-NT Release**

Both ambroxol and beclomethasone alone inhibited LPS-stimulated IL-8, MPO and 3-NT release by BEAS-2B cells cocultured with neutrophils (fig. 4). Ambroxol (10^{-5} M; 22.3% of inhibition) and beclomethasone (10^{-6} M; 24% of inhibition) similarly reduced LPS-induced IL-8 release in BEAS-2B cocultured with neutrophils, and the combination of both drugs additionally increased their effects (36% of inhibition, p < 0.05; fig. 4a). Moreover, ambroxol (10^{-5} M; 49% of inhibition) and beclomethasone (10^{-6} M; 57% of inhibition) similarly low-
ered LPS-induced MPO release in BEAS-2B cocultured with neutrophils, but the combination did not produce a further inhibition (48% of inhibition; fig. 4b). Finally, ambroxol \((10^{-7}\text{ M}; 24\%\text{ of inhibition})\) and beclomethasone \((10^{-6}\text{ M}; 26\%\text{ of inhibition})\) similarly inhibited LPS-induced 3-NT production in BEAS-2B cocultured with neutrophils, and the combination of both drugs significantly enhanced their inhibitory effects (41% of inhibition, \(p < 0.05\); fig. 4c).

**Effect of Ambroxol on p38 MAPK Phosphorylation**

LPS (1 \(\mu\text{g/ml}\) for 12 h) produced a marked increase in p-p38 in BEAS-2B cells (fig. 5). The addition of ambroxol \((10^{-6}\text{ M}; \text{in the last 4 h of incubation with LPS})\) almost completely suppressed p-p38 expression in BEAS-2B cells (fig. 5).

**Discussion**

The current study showed the ability of ambroxol and beclomethasone to exert neutrophil-related anti-inflammatory activity and to inhibit nitrosative stress in a bronchial epithelial cell model. In particular, ambroxol suppressed LPS-stimulated RANTES expression and IL-8 release, and additionally reduced iNOS expression. Furthermore, beclomethasone lowered LPS-stimulated iNOS expression and IL-8 release. In the presence of neutrophils, ambroxol and beclomethasone significantly decreased LPS-induced IL-8 and MPO release, and 3-NT formation. The combination of both drugs had an additive inhibitory effect on LPS-induced IL-8 release and 3-NT formation in bronchial epithelial cells cocultured with human neutrophils.
These findings support those in prior reports on the ability of LPS to provoke IL-8 production in bronchial epithelial cells [26, 27]. In our previous study we showed that bradykinin and LPS-induced bradykinin B2 receptor expression and nitrosative stress through the IL-8 pathway in BEAS-2B cells co-incubated with neutrophils, suggesting a role for bradykinin, via neutrophil activation, in the formation of nitrosative stress [4]. Moreover, both human rhinoviruses and house dust mites upregulated IL-8 release in BEAS-2B cells, while RANTES release was induced only by human rhinoviruses [28]. This means that the bronchial epithelium differently modulates airway inflammation on the basis of the exposure to specific stimuli. Another in vitro study showed that cocultures of BEAS-2B cells and eosinophils upregulated IL-8 and RANTES mRNA gene expression in BEAS-2B cells in

![Graph showing IL-8, MPO, and 3-NT production](image)

**Fig. 3.** IL-8 (a), MPO (b) and 3-NT (c) production at different time points (1, 2, 4 and 8 h) in BEAS-2B cells alone (BEAS), BEAS-2B cells cocultured with human neutrophils (BEAS + PMNs) and BEAS-2B cells cocultured with human neutrophils and treated with LPS 1 μg/ml (BEAS + PMNs + LPS). Results are expressed as mean nanograms per milliliter for IL-8 and MPO, and micrograms per milliliter for 3-NT. * p < 0.05, ** p < 0.01, *** p < 0.001.
conjunction with the sole release of IL-8, which was inhibited by nuclear factor (NF)-κB and p38 MAPK inhibitors [29], thus confirming that IL-8 release from epithelial cells is mediated through the p38 MAPK and NF-κB pathways [30, 31].

In addition, our data revealed increased RANTES protein expression induced by LPS in BEAS-2B cells, highlighting a link between RANTES and LPS-induced epithelial-cell-derived neutrophilic inflammatory response in the airways, as confirmed by a study on transgenic mice where RANTES overexpression caused increased neutrophil infiltration into the airways, thus demonstrating a role of RANTES in neutrophil recruitment into the airways [32]. Finally, RANTES expression in bronchial epithelial cells was shown to be regulated, at least in part, by the p38 MAPK pathway [33]. In a more recent study, we showed an increased number of CCL5/RANTES+ cells in bronchial epithelium and submucosa of patients with severe COPD in a stable phase, as well as an enhanced number and adhesiveness of neutrophils in the submucosa, supporting a role for RANTES in exaggerated bronchial mucosa neutrophilia of patients with severe COPD [34].

Our results for LPS-induced iNOS expression are in line with those from a recent study showing that induced sputum supernatants from COPD patients were able to increase iNOS expression and oxidative/nitrosative stress in human bronchial epithelial cells (16-HBE) compared to sputum supernatants from healthy controls and healthy smokers [35], indicating that iNOS is involved in epithelial-cell-derived nitrosative stress.

We demonstrated the ability of ambroxol and beclomethasone to differently regulate epithelial-cell-derived LPS-induced airway inflammation by inhibiting RANTES, iNOS or IL-8 expression, likely via Toll-like re-

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**Fig. 4.** IL-8 (a), MPO (b) and 3-NT (c) production at 8 h in BEAS-2B cells cocultured with human neutrophils (BEAS + PMNs), BEAS-2B cells cocultured with human neutrophils and treated with LPS 1 μg/ml (BEAS + PMNs + LPS), BEAS-2B cells cocultured with human neutrophils treated with LPS 1 μg/ml and ambroxol 10^{-5} M (BEAS + LPS + AMB), BEAS-2B cells cocultured with human neutrophils treated with LPS 1 μg/ml and beclomethasone 10^{-6} M (BEAS + LPS + BDP) and BEAS-2B cells cocultured with human neutrophils treated with LPS 1 μg/ml and ambroxol 10^{-5} M plus beclomethasone 10^{-6} M (BEAS + LPS + AMB + BDP). Results are expressed as mean nanograms per millilitre for IL-8 and MPO, and micrograms per millilitre for 3-NT. * p < 0.05, ** p < 0.01, *** p < 0.001.
Effect of Ambroxol and Beclomethasone on LPS-Induced Nitrosative Stress

Neutrophils are known to play a major role in the pathology of chronic obstructive respiratory diseases (COPD, chronic bronchitis and bronchiectasis) and are able to release chemokines such as IL-8 and RANTES as part of the inflammatory response [3, 34, 43–49]. MPO, the most abundant protein in neutrophils, plays a key role in neutrophil activation, catalyses the conversion of hydrogen peroxide (H$_2$O$_2$) and chloride ions into hypochlorous acid (HOCl), and is an important enzyme in the host defence against bacteria, viruses and fungi [50]. NO and nitrite (NO$_2^-$) are substrates of MPO in an inflammatory micro-environment in the presence of oxidative stress [3, 51, 52]. In vitro biochemical studies demonstrated that the nitration of protein tyrosine residues per se alters protein function and damages DNA, lipids and carbohydrates, leading to impaired cellular function and enhanced inflammatory reactions [1, 2].

In this study, an increase in IL-8, MPO and nitrosative stress, by means of 3-NT production, was evidenced in BEAS-2B cells cocultured with neutrophils compared to BEAS-2B cells alone [4]. Ambroxol and beclomethasone alone showed an inhibitory effect on LPS-induced IL-8 and MPO release and 3-NT formation in BEAS-2B cells cocultured with neutrophils, and the combination of the two drugs enhanced their effect on IL-8 and 3-NT. In other studies, ambroxol or beclomethasone were able to inhibit nitrosative stress [8, 13, 22], and ambroxol alone showed a great antioxidant activity, operating as scavenger of HClO and OH, and also revealed the capacity to decompose H$_2$O$_2$ in an in vitro study [12], but the combination has not previously been investigated in this context.
We also showed that ambroxol almost completely suppressed LPS-stimulated p-p38 expression in BEAS-2B cells. The interaction between nitrosative stress and the activation of the p38 MAPK pathway is a well-defined pathological mechanism in inflammatory disease [53–55], and the capability of ambroxol to prevent p38 phosphorylation, as shown in ours and in a previous study [56], suggests that this drug is able to inhibit the p38 MAPK signalling pathway in relation to nitrosative stress. Moreover, LPS inhalation in human volunteers induced activation of bronchial epithelium by increased expression of p38 MAPK and IL-8 [57], and the addition of p38 MAPK inhibitors reduced IL-8 expression in BEAS-2B stimulated by flagellin from P. aeruginosa [58], indicating that p38 inhibition might have a relevant role in reducing LPS-induced epithelial-cell-derived inflammation.

The strength of this study is that the ability of ambroxol to hinder neutrophil-related chemokines and markers of nitrosative stress in vitro has been clearly demonstrated, confirming other in vitro studies and supporting reports on these agents in the clinical setting, as well as the additive effect of ambroxol and beclomethasone in our model. This study has some limitations: as in other in vitro studies there is a difference between in vitro concentrations and in vivo doses; nevertheless, the tested concentration of beclomethasone is in line with that used in similar studies [22, 38, 59]. In this in vitro study, the described properties of ambroxol were observed at relatively high concentrations that may be reached only following inhaled administration of ambroxol, due to a more elevated amount of drug reaching the target site (topical administration). In addition, we have to consider that ambroxol is known to have high affinity to lung tissue, resulting in 16-fold higher tissue concentrations as compared to blood [60, 61]. Finally, in this study we did not use primary cultures from patients with COPD or other respiratory diseases. This might be a subject for future investigation.

In conclusion, the present study showed that ambroxol hydrochloride and/or beclomethasone dipropionate inhibited LPS-induced chemokine release and neutrophil-dependent nitrosative stress formation in an inflammatory model of human bronchial epithelial cells. The additive effect of ambroxol and beclomethasone on IL-8 and 3-NT production suggests new therapeutic options in the treatment of neutrophil-related chronic respiratory diseases such as chronic bronchitis/COPD and respiratory infections. Additional studies are warranted to confirm these effects in vivo, to further investigate the mechanisms of action of these drugs, and to evaluate their clinical efficacy and tolerability in patients with chronic inflammatory respiratory diseases in a stable condition and during exacerbation.

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Financial Disclosure and Conflicts of Interest

Drs. Ricciardolo, Sorbello and Benedetto have no conflicts of interest related to this publication. Dr. Paleari is an employee of Chiesi Farmaceutici SpA.

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