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Interaction between corticosteroids and muscarinic antagonists in human airways

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ABSTRACT

Background: To date there is emerging clinical evidence to add long-acting anti-muscarinic agents (LAMAs) with inhaled corticosteroid (ICSs) in asthma, but the pharmacological rationale that supports the use of such a combination has not yet been explained. The aim of this study was to pharmacologically investigate the interaction between the ICS beclomethasone and the LAMA glycopyrronium on the human airway smooth muscle (ASM) tone.

Methods: We investigated the rapid non-genomic bronchorelaxant effect of beclomethasone and glycopyrronium, administered alone and in combination, in human isolated bronchi and bronchioles. Experiments were carried out also in passively sensitized airways and the pharmacological analysis of drug interaction was performed by Bliss Independence method.

Results: The acute administration of beclomethasone and glycopyrronium induced a significant relaxation of passively sensitized ASM pre-contracted with histamine, by causing submaximal/maximal inhibition of the contractile tone in both medium bronchi and bronchioles. Beclomethasone was characterized by a rapid non-genomic and epithelium independent bronchorelaxant effect. In passively sensitized airways, this effect seemed to be dependent by the activation of a Gsα – cyclic adenosine monophosphate (cAMP) – protein kinase A cascade. While no synergistic interaction was detected in non-sensitized bronchi, the beclomethasone/glycopyrronium combination synergistically enhanced the relaxation of passively sensitized medium and small bronchi. The synergistic interaction between beclomethasone and glycopyrronium was associated with an increase of cAMP concentrations.

Conclusions: Our study provides for the first time the pharmacological rationale for combining low doses of an ICS plus a LAMA.

1. Introduction

Inhaled corticosteroids (ICSs) are the cornerstone of the treatment of asthma [1,2], but all national and international chronic obstructive pulmonary disease (COPD) guidelines and recommendations suggest ICSs only for patients with severe functional impairment and high risk of exacerbations despite an optimal regimen with a long-acting inhaled bronchodilator [3–6]. On the other hand, long acting muscarinic antagonists (LAMAs), although well established in COPD guidelines [3–6], are not currently licensed for asthma [1,2].

Nonetheless, there is emerging clinical evidence for the use of

Abbreviations: ANOVA, analysis of variance; ASM, airway smooth muscle; BHR, bronchial hyperresponsiveness; BI, Bliss Independence; cAMP, cyclic adenosine monophosphate; COPD, chronic obstructive pulmonary disease; CRC, concentration–response curve; E, effect; EC_n, concentration of an agonist that produces n% of the maximal possible effect of that agonist; E_{max}, maximal relaxant response; GOLD, Global Initiative for Chronic Obstructive Lung Disease; IC_{50}, concentration of an antagonist that reduces the response to an agonist by 50%; ICS, Inhaled corticosteroid; KH, Krebs-Henseleit; K_i, equilibrium dissociation constant; LABA, long acting β_2 agonist; LAMA, long acting muscarinic antagonists; PCLS, precision-cut lung slices; PKA, protein kinase A; PKC, protein kinase C; SEM, standard error of the mean.

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LAMAs in asthma when used in addition to ICS [7], and, besides, the Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommendations [5] suggest to add a LAMA to an ICS/long acting β_2 agonist (LABA) combination as second choice in those patients that have many symptoms and are at a high risk of exacerbations.

These guideline recommendations are based on clinical evidence, but the pharmacological rationale that can support the use of such a combination has not yet been explained. Consequently, there is a need to elucidate the potential interaction between corticosteroids and muscarinic receptors antagonists. These interactions may provide the pharmacological rationale for the use of LAMA/ICS as dual combination or as a part of triple combination therapy for the treatment of patients suffering from asthma or COPD.

Few experimental studies suggested that corticosteroids increase prejunctional auto-inhibitory M_2 muscarinic receptor on airway parasympathetic neurons, but these drugs decreased both M_2 and M_3 muscarinic receptors in airway smooth muscle (ASM) [8,9]. Moreover, corticosteroids inhibit the contractile effects of acetylcholine and vagal stimulation [10], and may influence the signal transduction pathways activated by both M_2 and M_3 muscarinic receptors. Furthermore, modulatory effects of corticosteroids on G-proteins, including G_i and G-proteins linked to K^+ channels, have been reported [11], although this evidence comes only from animal experimental models and cannot be extrapolated to humans [12].

In the present study we aimed to explore whether the combination of a corticosteroid and an anti-muscarinic agent is capable of eliciting an additive or even a synergistic interaction on human isolated airways, and to try to understand what could be the mechanism(s) behind this potential effect.

2. Materials and methods

2.1. Ethical approval and informed consent

Ethical approval and informed consent were obtained from the Istituto Regina Elena – Istituto San Gallicano (Rome, Italy) and they were consistent with the 2009 National Committee of Bioethics, National Committee of Bio-safety, Biotechnology and Sciences (Italy) recommendations on the collection of biologic samples for research purposes, the 2010 Italian ethical and legal recommendations concerning the biobank and the research biorepository (Istituto Nazionale dei Tumori – Independent Ethics Committee, 2010), and the Comitato Nazionale per la Biosicurezza, le Biotecnologie e le Scienze per la Vita (Raccolta di campioni biologici a fini di reicerca, consenso informato, 2009; available at: http://www.governo.it/bioetica/gruppo_misto/Consenso_Informato_allegato_Petrini_2009.pdf.

2.2. Preparation of tissues

Regions of macroscopically normal lungs were taken from uninvolved areas resected from 14 patients (8 male, 6 female; aged 63.3 ± 3.2 years) undergoing lobectomy for lung cancer, but without a history of chronic airway disease.

Tissue samples were immediately placed into oxygenated Krebs-Henseleit (KH) buffer solution (NaCl 119.0 mM, KCl 5.4 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 25.0 mM, glucose 11.7 mM; pH 7.4) containing the cyclooxygenase inhibitor indometacin (5.0 μM), and transported at 4 °C from the Regina Elena National Cancer Institute (Rome, Italy) to the Laboratory of Clinical Respiratory Pharmacology at the Medical School of the University of Rome "Tor Vergata" (Rome, Italy). None of the patients had been chronically treated with theophylline, β_2-agonists, anti-muscarinic agents or corticosteroids. Serum immunoglobulin E levels determined on the day of surgery were in the normal range. Preoperative lung function parameters were generally normal and there were no signs of respiratory infections.

In the laboratory, airways were dissected from connective and alveolar tissues and refrigeration overnight in KH buffer solution. The next morning, bronchi were cut into rings (medium airways, segmental bronchi; thickness: 1–2 mm; diameter: 4–6 mm) and transferred into a 10 ml High Tech 8 Channels Manual Compact Organ Bath system (Panlab Harvard Apparatus, Spain) containing KH buffer solution (37 °C) and continuously aerated with O_2/CO_2 (95:5%).

Precision-cut lung slices (PCLSs) were sectioned (small airways, bronchioles; thickness: <500 μm; diameter: 0.89 ± 0.08 mm) by using a Vibroslice Microtome equipped with ceramic blades (Campden Instruments, UK). Slices were processed without the complications related to the use of confounding agarose gel to inflate the lung or complex parenchymal sections that have numerous contracting elements [13–15]. PCLSs were mounted into a Visual Imaging and Patching Chamber connected to a Proportional Integral Derivative Temperature Controller with dual thermistor feedback CI7800 (Campden Instruments, UK), containing KH buffer solution (37 °C) and continuously aerated with O_2/CO_2 (95:5%).

2.3. Passive sensitization

The passive sensitization of isolated bronchi is a model that closely mimics important characteristics of bronchial hyperresponsiveness (BHR) *in vivo*. Therefore, this model was used to study the acute anti-spasmogenic effect of beclomethasone dipropionate (only "beclomethasone" afterward in the manuscript), alone or in combination with glycopyrronium bromide (only "glycopyrronium" afterward in the manuscript), in human hyperreactive bronchi [16]. Human isolated bronchial tissues were rotated overnight at room temperature in tubes containing KH buffer solution in the absence (non-sensitized control bronchi) or the presence of 10% vol^{-1} sensitizing serum (sensitized bronchi). Sera were prepared by centrifugation from the whole blood of patients suffering from atopic asthma (total IgE >1000 U ml^{-1} specific against common aeroallergens) during exacerbation [17,18] providing signed consent for serum donation. Sera were frozen at −80 °C in 250 μl aliquots until required. The next morning bronchial tissues were transferred into the organ bath or PCLS system containing KH buffer solution (37 °C) and continuously gassed with a 95% O_2/5% CO_2.

2.4. Epithelium removal

The bronchial epithelium was mechanically removed using a cotton-tipped applicator gently rubbed for 5 s on the luminal surface as previously described [19–21]. It has been previously demonstrated that this manipulation does not penetrate the basal membrane and that the lamina propria remains almost intact [22]. Epithelium removal and the integrity of surrounding bronchial layers were confirmed by histology.

2.5. Measurement of bronchial smooth muscle contraction

Each bronchial ring was connected to an isometric force transducer (Fort25; WPI, UK) and allowed to equilibrate for 90 min before being flushed with fresh KH buffer solution every 10 min. The contractile signal was amplified by a Powerlab 8/36 and Octal Bridge Amp system (AD instruments, UK) and recorded and analyzed by using LabChart 7 interface software (AD instruments, UK). Passive tension was determined by gentle stretching of the tissue (0.5–1.0 g) during equilibration. The transducer measured