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Pharmacological characterization of the interaction between aclidinium bromide and formoterol fumarate on human isolated bronchi

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ABSTRACT

Long-acting muscarinic receptor antagonists (LAMAs) and long-acting β_2-adrenoceptor agonists (LABAs) cause airway smooth muscle (ASM) relaxation via different signal transduction pathways, but there are limited data concerning the interaction between these two drug classes on human bronchi. The aim of this study was to investigate the potential synergistic interaction between aclidinium bromide and formoterol fumarate on the relaxation of human ASM. We evaluated the influence of aclidinium bromide and formoterol fumarate on the contractile response induced by acetylcholine or electrical field stimulation (EFS) on human isolated airways (segmental bronchi and bronchioles). We analyzed the potential synergistic interaction between the compounds when administered in combination by using Bliss independence (BI) theory. Both aclidinium bromide and formoterol fumarate completely relaxed segmental bronchi pre-contracted with acetylcholine (E_{max}: 97.5 ± 2.6% and 96.4 ± 1.1%; pEC_{50} 8.5 ± 0.1 and 8.8 ± 0.1; respectively). Formoterol fumarate, but not aclidinium bromide, abolished the contraction induced by acetylcholine in bronchioles (E_{max}: 68.1 ± 4.5% and 99.0 ± 5.6%; pEC_{50} 7.9 ± 0.3 and 8.4 ± 0.3; respectively). The BI analysis indicated synergistic interaction at low concentrations in segmental bronchi (+18.4 ± 2.7%; $P<0.05$ versus expected effect) and from low to high concentrations in bronchioles (+19.7 ± 0.9%; $P<0.05$ versus expected effect). Low concentrations of both drugs produced a synergistic relaxant interaction on isolated bronchi stimulated with EFS that was sustained for 6 h post-treatment (+55.1 ± 9.4%; $P<0.05$ versus expected effect). These results suggest that combining aclidinium bromide plus formoterol fumarate provides synergistic benefit on ASM relaxation of both medium and small human airways, which may have major implications for the use of this combination in the clinic.

1. Introduction

Bronchodilators are crucial for the management of symptoms of chronic obstructive pulmonary disease (COPD) and asthma (GINA, 2012; GOLD, 2014). There are currently two classes of long-acting bronchodilators with different pharmacological mechanisms: muscarinic receptor antagonists agents and β_2-adrenoceptor agonists. Long-acting muscarinic receptor antagonist (LAMAs) and long-acting β_2-adrenoceptor agonists (LABAs) are used for regular treatment of COPD, whereas short-acting muscarinic receptor antagonists agents and

Abbreviations: β_2-AR, β_2-adrenoreceptor; ASM, airway smooth muscle; BI, Bliss independence; COPD, chronic obstructive pulmonary disease; CRC, concentration-response curve; E, effect; EFS, electrical field stimulation; EC_n, effective concentration for n% of maximal effect; E_{max}, maximal effect; FEV_1, forced expiratory volume in 1 s; GINA, Global Initiative for Asthma; GOLD, Global Initiative for Chronic Obstructive Lung Disease; KH, Krebs-Henseleit; LAMA, long-acting antimuscarinic; LABA, long-acting β_2-adrenoceptor agonist; pEC_{50}, the negative logarithm of EC_{50}; PCLS, precision-cut lung slices

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short-acting β_2-adrenoceptor agonists are used as rescue medications for acute treatment of airways obstruction.

Combining a LAMA with a LABA might be a valuable therapeutic approach for maintenance treatment of patients with stable-moderate COPD whose symptoms are not adequately controlled with monotherapy using the LAMA tiotropium bromide (Rodrigo et al., 2012). Furthermore, it has been suggested that fixed-dose combination therapy with two classes of bronchodilator in the same inhaler would simplify treatment regimens and improve patient adherance (Cazzola and Matera, 2008).

A twice-daily fixed-dose combination of the LAMA aclidinium bromide and the LABA formoterol fumarate is currently under clinical development for the treatment of COPD. The Phase III ACLIFORM COPD study showed that aclidinium bromide/formoterol fumarate 400/6 μg and 400/12 μg significantly improved 1-h post-dose forced expiratory volume in 1 s (FEV_1) versus aclidinium bromide monotherapy and trough FEV_1 versus formoterol fumarate monotherapy at Week 24 (Singh et al., 2014). Both parameters were significantly improved versus placebo and the findings of a second Phase III study – AUGMENT COPD – were similar (D'Urzo et al., 2014).

Although these studies suggest that combining aclidinium bromide with formoterol fumarate may be clinically useful, the full extent of the interaction between these compounds is not yet well understood. As LABAs and LAMAs both cause airways smooth muscle (ASM) relaxation via different signal transduction pathways, combining a LABA plus a LAMA might prove beneficial for a number of reasons (Cazzola and Molimard, 2010). For example, LABAs decrease the release of acetylcholine through modulation of cholinergic neurotransmission by acting on prejunctional β_2-adrenoreceptors (β_2-ARs) leading to the activation of calcium-activated potassium channels that hyperpolarize the cell membrane, which amplifies the ASM relaxation induced by the LAMA. Furthermore, LAMAs antagonize the bronchoconstrictor effects of acetylcholine, whose release can be modified by the LABA, which may amplify the bronchodilation induced by the LABA through the direct stimulation of ASM β_2-ARs (Cazzola and Molimard, 2010; Cazzola et al., 2013). In addition, crosstalk between G_q-coupled M_3 receptors and G_s-coupled β_2-ARs may influence the β-agonist-induced relaxation, possibly by activation of protein kinase C (PKC) and subsequent phosphorylation of β_2-AR and/or G_s protein (Cazzola et al., 2013).

Although there is clear scientific rationale for combining LAMAs and a LABAs in the treatment of COPD, to date there are limited pharmacologic data investigating the interaction between these drugs at the level of human bronchi. Therefore, the aim of this study was to investigate the pharmacological interaction between aclidinium bromide and formoterol fumarate on the relaxation of human segmental bronchi. In addition, small airways were evaluated in order to better understand the interaction between these drugs on ASM found at different anatomical levels of human respiratory tract.

2. Material 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 biological samples for research purposes, the 2010 Italian ethical and legal recommendations concerning the biobank, and the research biorepository (Istituto Nazionale dei Tumore – 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 23 patients (14 male, 9 female; aged 63.1 ± 2.2 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 indomethacin (5.0 μM), and transported at 4 °C from the Regina Elena National Cancer Institute (Rome, Italy) to the Laboratory of Respiratory Pharmacology in 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, or glucocorticosteroids. 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 refrigerated 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: 0.93 ± 0.08 mm) 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 (Calzetta et al., 2014b; van Lunteren and Moyer, 2001; Wohlsen et al., 2001). 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. Preparation of drugs

Test compounds were maintained under dry conditions and prepared daily. Stock solutions used in this study were: acetylcholine (Sigma-Aldrich, Italy), papaverine (Sigma-Aldrich, Italy), indomethacin (Sigma-Aldrich, Italy), formoterol fumarate (a kind gift from Almirall, Spain), and aclidinium bromide (a kind gift from Almirall, Spain). All products were dissolved in distilled water, except aclidinium bromide, which was dissolved in 1N HCl 1% (v/v). Indomethacin was dissolved in pure ethanol and diluted in KH buffer solution prior to use. The maximal concentration of ethanol used to prepare drug solutions (0.02%) did not influence isolated tissue responses as reported elsewhere (Freas et al., 1989; Hatake and Wakabayashi, 2000). Appropriate dilutions were obtained in freshly prepared medium. Stock solutions were stored at −80 °C until use.

2.4. Measurement of bronchial smooth muscle contraction

Each bronchial ring was connected to an isometric force transducer (Fort25; WPI, UK) and was allowed to equilibrate for 90 min before being flushed with fresh KH buffer solution every 10 min. The signal was amplified by a Powerlab 8/36 and Octal Bridge Amp system (AD instruments, UK) and recorded and