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Searching for the synergistic effect between aclidinium and formoterol: From bench to bedside

Mario Cazzola [a, b, c, *], Luigino Calzetta [b], Josuel Ora [c], Ermanno Puxeddu [a, c], Paola Rogliani [a, c], Maria Gabriella Matera [d]

[a] University of Rome Tor Vergata, Department of Systems Medicine, Chair of Respiratory Medicine, Rome, Italy
[b] University of Rome Tor Vergata, Department of Systems Medicine, Respiratory Pharmacology Research Unit, Rome, Italy
[c] University Hospital Tor Vergata, Division of Respiratory Medicine, Rome, Italy
[d] Second University of Naples, Department of Experimental Medicine, Unit of Pharmacology, Naples, Italy

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ABSTRACT

Aim of our study was to understand if the interaction between aclidinium and formoterol administered at therapeutic doses leads to a synergistic rather than additive broncholytic effect. We tested the type of effect *ex vivo* on isolated human bronchi and then *in vivo* in COPD patients. The analysis of the interaction between aclidinium and formoterol *in vitro* was measured by applying the Unified Theory, whereas that in COPD patients was measured by applying the Bliss Independence criterion. Aclidinium and formoterol administered alone completely relaxed human isolated bronchial tissues sub-maximally pre-contracted with ACh in a concentration-dependent manner with similar potency (EC_{50}: aclidinium 4.64 ± 0.78 nM, formoterol 2.71 ± 0.21), whereas the interaction of aclidinium plus formoterol produced moderate to strong synergism. Changes in FEV_1 values showed that inhaled aclidinium and formoterol induced a significant and time-dependent bronchodilatory effect during the study time. The inhalation of aclidinium and formoterol in combination significantly anticipated at 5 min post-administration the bronchodilatory effect of FEV_1, compared with the effect of drugs administered alone. There was a synergistic interaction for FEV_1 at 5 min and from 120 min to 240 min post-inhalation, whereas from 30 min to 60 min post-administration the drug interaction was additive. This study shows that aclidinium and formoterol can produce a significant synergistic interaction that may have a role also in the clinic setting.

1. Introduction

There is a validated clinical rationale for combining β_2-agonists and antimuscarinic agents [1,2], although the nature (additive or synergistic) of the results obtained by the co-administration of β_2-agonists and antimuscarinic agents is not fully clarified by the pharmacological point of view [3].

Recently, we evaluated the influence of aclidinium bromide and formoterol fumarate on the contractile response induced by acetylcholine (ACh) and electrical field stimulation (EFS) on human isolated airways and observed that the combination produced a synergistic interaction that induced a considerably faster onset of action on the reduction of the EFS-induced contractile tone [4].

Isolated airway preparations provide a suitable, convenient, and reproducible biological model for the evaluation of drug effects on airway smooth muscle (ASM) independent from the potential systemic influences found *in vivo*. Unfortunately, however, the response of isolated human ASM may not always be identical to that elicited *in vivo*. In fact, studies on isolated bronchial ASM strips alone may inherently neglect many interactions that occur at larger length scales and that can impact ASM contractility and resultant airway narrowing [5].

In order to understand if the interaction between aclidinium and formoterol administered at therapeutic doses leads to a synergistic rather than additive broncholytic effect even in COPD patients, we have explored whether the acute bronchodilation induced by the free combination of 322 μg aclidinium and 9 μg formoterol is additive or synergistic with respect to monocomponents by testing the type of effect *ex vivo* on isolated human bronchi and then *in vivo* in patients with COPD.

* Corresponding author. Università di Roma Tor Vergata, Dipartimento di Medicina dei Sistemi, Via Montpellier 1, 00133 Rome, Italy.
E-mail address: mario.cazzola@uniroma2.it (M. Cazzola).

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2. Materials and methods

2.1. Ex vivo study

2.1.1. Preparation of tissues

Regions of macroscopically normal lungs were taken from uninvolved areas resected from 8 patients (4 males and 4 females, 65.2 ± 1.3 years old) undergoing lobectomy surgery for lung cancer, but without a history of chronic airway disease.

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 ricerca, consenso informato, 2009; available at: http://www.governo.it/bioetica/gruppo_misto/Consenso_Informato_allegato_Petrini_2009.pdf).

Airways were immediately placed into oxygenated Krebs-Henseleit buffer solution (KH) (mM: NaCl 119.0, KCl 5.4, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 11.7; pH 7.4) containing the cyclooxygenase (COX) inhibitor indomethacin (5.0 μM), and transported at 4 °C from IRE-ISG to the Laboratory of Respiratory Clinical Pharmacology (University of Rome Tor Vergata, Rome, Italy). None of the patients were chronically treated with theophylline, β_2-agonists or corticosteroids. Serum IgE 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. Then, segmental bronchi were isolated and stored overnight in KH buffer solution at refrigeration temperature. The next morning, bronchi were cut into rings (thickness: 1–2 mm; diameter: 5–7 mm) and transferred into 4400 four-chamber 10 ml isolated organ baths (Ugo Basile, Varese, Italy) containing KH buffer (37 °C) and continuously aerated with a 95:5% mixture of O_2/CO_2.

2.1.2. Preparation of drugs

The following drugs were used: acetylcholine (ACh), aclidinium, formoterol, indomethacin and papaverine. All substances were obtained from Sigma–Aldrich (St. Louis, USA). Drugs were dissolved in distilled water except for indomethacin, which was dissolved in ethanol and then diluted in a KH buffer. The maximal amount of ethanol (0.02%) did not influence isolated tissue responses [6,7]. Compounds were stored in small aliquots at −80 °C until their use.

2.1.3. Tension measurement

Human bronchi were placed in organ baths containing KH buffer solution (37 °C) medicated with indomethacin (5.0 μM), bubbled with 95%O_2/5%CO_2 and suspended under passive tension (0.5–1.0 g). Bronchial rings were mounted on hooks in the organ baths where one hook was attached with thread to a stationary rod and the other hook tied with thread to an isometric force displacement transducer. Airways were allowed to equilibrate for 90 min with repeated changes of the medicated KH buffer solution every 10 min. Changes in isometric tension were measured with a transducer (Fort 10 WPI, Basile, Instruments, Varese, Italy) and the tissue responsiveness was assessed by measuring the ASM response to ACh (100 μM); when the contractile response reached a plateau, rings were washed three times and allowed to equilibrate for 45 min.

2.1.4. Study design

In order to test the potential synergistic relaxant interaction induced by aclidinium plus formoterol, the bronchial rings were contracted with ACh at the concentration required to cause a 70% maximal effect (EC_{70}). In fact, although in lung of COPD patients several pathways may influence the bronchial tone, it has been so far and widely recognized that the cholinergic signaling is the main pathway altered in COPD [8]. After that, concentration response curves (CRCs) were constructed to test aclidinium and formoterol alone or administered in combination at constant-ratio drug combination, as described elsewhere [9]. Intervals of 5–15 min between successive concentrations were used to reach a stable level of relaxation before the administration of the next concentration.

At the completion of the experiments, papaverine (500 μM) was added to relax the tissues completely and provide a standard relaxant effect to which the relaxation of each tissue could be compared.

2.1.5. Analysis of results

Appropriate curve-fitting to a sigmoidal model was used to calculate the effect (E), the Emax and the concentration required to cause 50% and 70% maximal effect (EC_{50} and EC_{70}, respectively). The equation used was log[agonist] vs. response, Variable slope, expressed as Y=Bottom + (Top-Bottom)/{1 + $10^{\wedge}$[(LogEC50-X) *HillSlope]}. E/Emax was expressed as percentage of Emax elicited by the contractile agents and the relaxant responses were expressed as a percentage of papaverine (500 μM) induced relaxation [10,11].

The analysis of the interaction between aclidinium and formoterol was measured by applying the Unified Theory, as described elsewhere [9]. Briefly, the Unified Theory is represented by the Median-Effect equation that includes four major biochemical and biophysical equations (Henderson-Hasselbalch, Michaelis–Menten, Hill, and Scatchard), leading to the Combination Index theorem and to the isobologram equation. Thus, the Combination Index is effect-oriented and quantifies the synergism or antagonism, where values <1, = 1, and >1 indicate synergism, additive effect and antagonism, respectively, whereas the isobologram is dose-oriented and is expressed as a graph with equipotency sum of doses [9].

All values are presented as mean ± SEM of different experiments (n = 3) and statistical significance was assessed by Student's t-test. The level of statistical significance was defined as $P < 0.05$ [12]. All data analyses were performed using computer software (GraphPad Prism 5, San Diego, CA, USA and CompuSyn, Paramus, NJ. USA).

2.2. Clinical trial

2.2.1. Inclusion criteria

The inclusion criteria for the clinical trial were A) male and female subjects, B) age >50 years old, C) diagnosis of COPD in agreement with ATS/ERS guidelines [13]; D) current or former smokers (>20 pack years) and E) moderate to severe COPD by FEV_1 of 35–80% predicted.

2.2.2. Exclusion criteria

The exclusion criteria were A) any exacerbation of COPD or respiratory diseases within four weeks of the screening visit, B) asthma according to GINA guidelines [14] and any further chronic respiratory disease excluded COPD, C) atopy and D) any contraindications to the use of aclidinium, formoterol and salbutamol according to the product label.

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