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Drug interaction and chronic obstructive respiratory disorders

Paola Rogliani [a,b,*], Beatrice Ludovica Ritondo [a], Bartolomeo Zerillo [b], Maria Gabriella Matera [c], Luigino Calzetta [d]

[a] Unit of Respiratory Medicine, Department of Experimental Medicine, University of Rome "Tor Vergata", Rome, Italy
[b] Division of Respiratory Medicine, University Hospital "Policlinico Tor Vergata", Rome, Italy
[c] Unit of Pharmacology, Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy
[d] Department of Medicine and Surgery, Respiratory Disease and Lung Function Unit, University of Parma, Parma, Italy

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ABSTRACT

Chronic obstructive respiratory disorders uncontrolled by monotherapy should be given combinations of drugs that act by distinct mechanisms of action. The rationale for combining different classes of drugs should be to elicit a synergistic interaction, lower the dose of the single components in the combinations and, thus, reduce the risk of adverse events.

The aim of this systematic review was to investigate the combined effect of drugs acting on human airways, by including studies that used a validated method for assessing the nature of drug interaction.

Current evidence indicates that drug combinations modulating the bronchial contractility induce a synergistic relaxant effect when the individual components are combined at isoeffective concentrations. There are several mechanisms of action underlying drug interactions. Pharmacological research has been directed to elucidate what causes the synergism between long-acting β_2-adrenoceptor (β_2-AR) agonists (LABAs), long-acting muscarinic antagonist (LAMAs), and inhaled corticosteroids (ICS) administered as dual or triple combination. Conversely, the mechanisms behind the additive interaction between phosphodiesterase 3 and 4 inhibitors and LAMAs, and the synergistic interaction between proliferator-activated receptor gamma ligands and β_2 agonists have been only hypothesized. Overall, the synergism elicited by combined drugs for the treatment of chronic respiratory disorders is an effect of class, rather than specific for drug combinations. Optimal synergy can be achieved only when the single agents are combined at isoeffective concentrations, and when monocomponents are given concurrently to reach together the same levels of the bronchial tree.

1. Introduction

Patients suffering from chronic obstructive respiratory disorders, such as asthma and chronic obstructive pulmonary disease (COPD), that remain uncontrolled by monotherapy, should be given combinations of drugs that act by different mechanisms of action (van der Molen and Cazzola, 2012; Quirce et al., 2015). Combining different classes of drugs may lead to three main types of interaction, namely the synergistic, antagonistic, and additive effect. By definition, synergy is an interaction greater than the expected additive effect, while antagonism is the observed effect being less than additive (Calzetta et al., 2018a). The first step to assess the presence of synergy and/or antagonism is to quantify the additive effect. In this respect, both the Bliss Independence criterion and the Unified Theory represent validated methods to identify the additive effect of drugs active on the respiratory system, but only the Bliss approach is suitable to provide statistical significance (Calzetta et al., 2015a).

The rationale for combination therapy is to elicit a synergistic interaction across monocomponents, a way to optimize the efficacy of agents and, eventually, reduce the doses of drugs and the risk of adverse events (Calzetta et al., 2018a). In any case, particular attention should be paid to avoid overlapping drug toxicities (Chou, 2006).

Since to date the pharmacological interaction (synergy, antagonism, additivity) of combination therapy has never been investigated, the aim of this review was to systematically assess interaction nature of combination therapies active at the level of human airways.

* Corresponding author. Department of Experimental Medicine, University of Rome "Tor Vergata", Via Montpellier, 1-00133, Rome, Italy.
E-mail address: paola.rogliani@uniroma2.it (P. Rogliani).

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2. Materials and methods

2.1. Review question

The question of this systematic review was to evaluate the nature of the pharmacological interaction of combination therapies active on human airways and used for the treatment of chronic obstructive respiratory disorders.

2.2. Search strategy and study eligibility

This synthesis of the current literature was performed in agreement with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) (Moher et al., 2015), with the relative flow diagram shown in Fig. 1. This study satisfied all the recommended items reported by the PRISMA-P checklist (Moher et al., 2015). The PICO (Patient problem, Intervention, Comparison, and Outcome) framework was applied to develop the literature search strategy and question, as previously reported (Schardt et al., 2007). Namely, the "Patient problem" included chronic obstructive respiratory disorders; the "Intervention" regarded dual or triple combination therapies; the "Comparison" was performed with respect to the monocomponents and/or expected additive effect; the assessed "Outcome" was the nature of drug interaction elicited on human airways (synergy, antagonism, additivity).

A comprehensive literature search was performed for studies (in vitro, ex vivo, and clinical trials) written in English, and characterizing the pharmacological interaction between drugs active on human airways and used to treat chronic obstructive respiratory disorders. A key inclusion criterion for the selection of the studies was the use of a validated pharmacological model for assessing and/or quantifying the drug interaction. The search was performed in MEDLINE in order to provide for relevant studies available with no time limit up to September 3rd, 2020. The research string was as follows: (asthma OR COPD) AND (bronchi OR airways) AND bronchodilation AND (interaction OR synergy). Citations of previous published relevant and recently published reviews were examined to select further pertinent studies, if any (Calzetta et al., 2018b). Two reviewers independently checked the relevant studies identified from the literature search. The studies were selected in agreement with previously mentioned criteria and any difference in opinion about eligibility was resolved by consensus.

2.3. Data extraction

Data from included studies were extracted in agreement with Data Extraction for Complex Meta-anALysis (DECiMAL) recommendations (Pedder et al., 2016).

2.4. Endpoints

The endpoint of this systematic review was to investigate the nature of pharmacological interaction (synergy, antagonism, additivity) across drugs active on human airways and used for the treatment of chronic obstructive respiratory disorders.

2.5. Strategy for data analysis

Data from original papers were extracted and reported via qualitative synthesis.

3. Results

Of the 402 potentially relevant records identified in the initial search, 13 studies were deemed eligible for a qualitative analysis.

Overall, this systematic review included data obtained from pre-clinical studies and/or clinical trials performed on COPD patients: 8 studies were conducted ex vivo in human isolated bronchial tissue collected from subjects without a history of chronic obstructive airway disease (Cazzola et al., 2014, 2016a, 2016b; Calzetta et al., 2013, 2015b, 2017a, 2018c, 2019a), 2 studies were carried out both ex vivo on human isolated bronchial tissue and in vivo in COPD patients (Cazzola et al., 2015a, 2015b), 1 study was performed ex vivo in human isolated bronchial tissue collected from COPD patients (Rogliani et al., 2020a), 1 study was a pooled analysis of 2

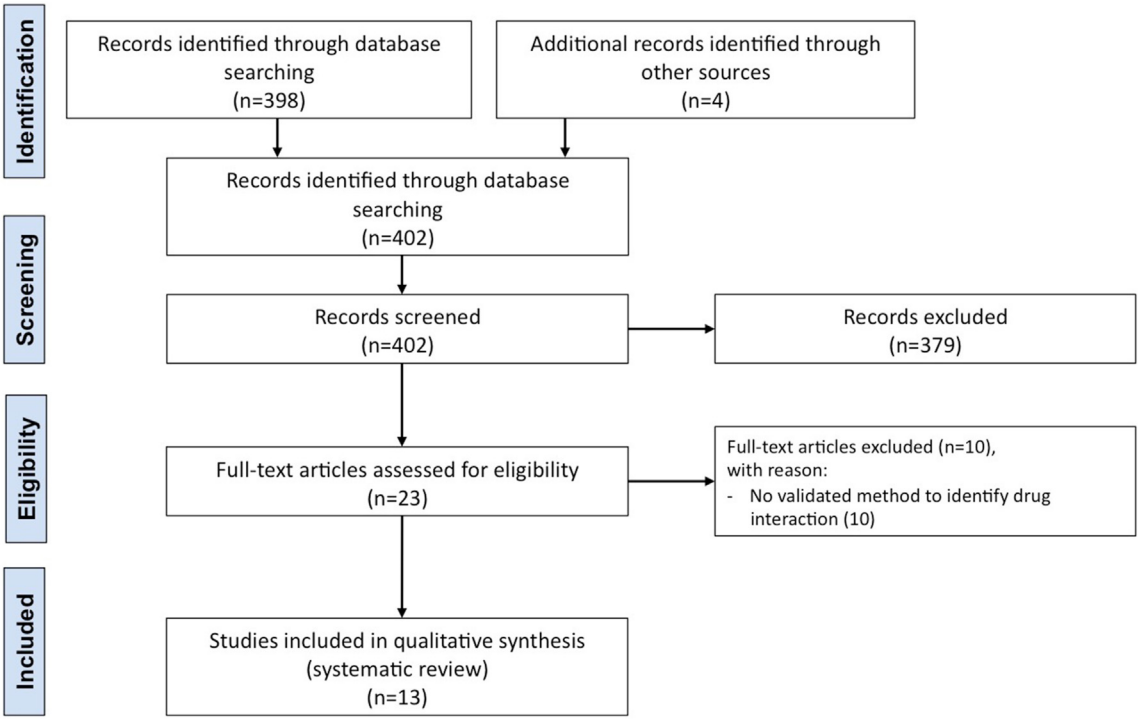

Fig. 1. PRISMA flow diagram for the identification of the studies included in the systematic review concerning synergistic drug interaction in chronic obstructive pulmonary disorders. PRISMA, Preferred Reporting Items for Systematic Review and Meta-Analysis.

randomized controlled trials on moderate-to-very-severe COPD patients (Donohue et al., 2016), and 1 study was performed in vitro human bronchial airway smooth muscle (ASM) cells (Fogli et al., 2013).

3.1. Dual combination

3.1.1. Clinical trials

The long-acting β_2-adrenoceptor (AR) agonist (LABA) indacaterol (IND) 50 μg and the long-acting muscarinic antagonist (LAMA) glycopyrronium bromide (GLY) 150 μg administered as free combination to COPD patients induced an additive bronchorelaxant effect leading to increased forced expiratory volume in the 1st second (FEV_1) between 5 min and 3 h post-inhalation, and synergistic interaction was detected only 15 min after the administration of the combination (Cazzola et al., 2015a).

The LABA formoterol fumarate (FF) 9 μg and the LAMA aclidinium bromide (ACL) 322 μg administered as free combination increased FEV_1 in COPD patients, by reaching the maximal effect at 120 min post-inhalation (change from pre-dosing of FEV_1: 137.62 ± 15.98 mL) (Cazzola et al., 2015b). FF/ACL elicited a stable synergistic interaction on FEV_1 at 5 min and between 120 min and 240 min post-inhalation (Cazzola et al., 2015b). The maximal synergistic response of +55.14 ± 14.34% was achieved 5 min post-administration compared with the effect of monocomponents, whereas between 30 min and 60 min post-inhalation, the pharmacological interaction was additive (Cazzola et al., 2015b).

The combination between the LABA vilanterol (VI) 25 μg and the LAMA umeclidinium bromide (UMEC) 62.5 μg improved the change from baseline in trough FEV_1 by 263 mL in moderate-to-severe COPD patients (Donohue et al., 2016). The interaction between VI 25 μg and UMEC 62.5 μg with respect to trough FEV_1 was less than fully additive in dual responders, fully additive in responders to the single monocomponents, and more than fully additive in the non-responders (Donohue et al., 2016).

3.1.2. Pre-clinical studies

3.1.2.1. Interaction between β_2-AR agonists and muscarinic antagonists. In medium bronchi, IND/GLY induced a synergistic relaxant response to acetylcholine (ACh) already at low concentrations (IND: 0.1 nM–40.0 nM, GLY: 0.4 nM–3.4 nM) and the maximal improvement in the bronchorelaxant response was +32.51 ± 7.86% compared to the expected additive effect (Cazzola et al., 2016b). When administered at isoeffective concentrations eliciting 20% of the maximal effect (E_{max}) (EC_{20}), IND/GLY produced an additive effect in medium bronchi pre-contracted by ACh, whereas at isoeffective concentrations inducing 30% of E_{max} (EC_{30}) the interaction was synergistic (delta effect: 0.26 ± 0.03 compared to the expected relaxant response) (Cazzola et al., 2015a). In medium bronchi pre-contracted by histamine (His), IND/GLY induced an additive effect (Cazzola et al., 2016b).

Isoeffective mixture (EC_{20}) of low concentrations of IND/GLY synergistically relaxed the bronchial contractile tone induced by electrical field stimulation (EFS) at 10 Hz (EFS_{10Hz}) (Cazzola et al., 2016b). The drug mixture produced a maximal relaxant effect of 58.82 ± 15.32%, which increased up to 71.95 ± 2.37% at ≃3 h post-administration and the bronchorelaxation remained stable up to 12 h (Cazzola et al., 2016b). The maximal synergistic relaxant response on the transmural stimulation was +32.18 ± 5.44% greater than the expected additive effect (Cazzola et al., 2016b).

IND/GLY synergistically relaxed small airways pre-contracted by Ach at low concentrations (IND: 0.03 μM–0.13 μM, GLY: 0.2 nM–1.5 nM) and produced an increased relaxant response of +28.46 ± 5.35% compared to the expected additive effect (Cazzola et al., 2016b).

FF/ACL synergistically relaxed medium bronchi pre-contracted by ACh already at low concentrations (FF: 0.84 nM–1.20 nM, ACL: 1.15 nM–2.15 nM) (Cazzola et al., 2014), and the maximal increase achieved in the bronchorelaxant response was +18.37 ± 2.72% compared to the expected additive effect (Cazzola et al., 2014). The analysis of drug interaction confirmed that at isoeffective concentrations inducing EC_{30}, FF/ACL produced a synergistic relaxant effect of 69.31 ± 2.59%, whereas at higher concentrations no evidence of synergism was observed (Cazzola et al., 2014). Moderate to strong synergism was detected in the range of isoeffective concentrations 0.5 nM−10 nM for FF and 0.6 nM–90 nM for ACL (Cazzola et al., 2015b). These results were confirmed in isobologram studies and by the values of Combination Index (CI) ranging from 0.131 to 0.142 (CI values < 1 indicate synergism) (Cazzola et al., 2015b). In medium bronchi contracted by EFS_{10Hz}, FF/ACL administered at isoeffective concentrations inducing EC_{20} produced a maximal relaxation of 69.74 ± 6.35%, which increased up to 82.36 ± 2.54% at ≃3.3 h post-administration (Cazzola et al., 2014). Low concentrations of FF/ACL induced a synergistic relaxant effect that was sustained for 6 h post-treatment (Cazzola et al., 2014). The maximal increase in the bronchorelaxant response was +55.12 ± 9.37% greater than the expected additive response and it was achieved 1.4 h post-administration (Cazzola et al., 2014). FF/ACL also synergistically relaxed small airways pre-contracted by ACh (FF 1.8 nM–63.0 nM, ACL 3.2 nM–1.0 μM), leading to a maximal bronchorelaxant response of +19.67 ± 0.85% compared to the additive effect (Cazzola et al., 2014). In small airways, the interaction between FF and ACL produced a luminal area enhancement of 69.89 ± 2.28% compared to the monocomponents (Cazzola et al., 2014).

VI/UMEC administered at the ratio of concentrations 22:55 reproducing that of the currently approved fixed-dose combination (FDC) (US Food and Drug Administration, 2016), inhibited the contractile response induced by $EFS_{1–50Hz}$ in medium bronchi, leaving a residual contractility of 13.23 ± 9.07% compared to monocomponents (Calzetta et al., 2017a). VI/UMEC administered at 22:55 concentration-ratio completely relaxed the bronchial tone and reached an E_{max} at 10 Hz of 99.6 ± 8.0% (Calzetta et al., 2017a). Nevertheless, when administered at low to high concentrations, VI plus UMEC produced an additive, rather than synergistic bronchorelaxant effect on the contractile tone induced by $EFS_{3–25Hz}$ (Calzetta et al., 2017a). Indeed, it was demonstrated that at the concentration-ratio 22:55, VI resulted to be under-dosed and UMEC over-dosed for EC_{25-75}, and the concentrations in the drug combinations were rather balanced just for an additive effect at EC_{90}, with no evidence of a synergistic interaction (Calzetta et al., 2017a). On the contrary, VI plus UMEC administered at low to very low isoeffective concentrations, different than the concentration-ratio 22:55, induced a strong to very strong synergistic relaxation of medium bronchi contracted by $EFS_{3–25Hz}$ (VI: 68.75 nM–137.50 nM, UMEC: 0.23 nM–0.47 nM) (Calzetta et al., 2017a). The maximal improvement of relaxant response elicited by the drug mixture was +41.40 ± 5.81% compared to monocomponents, leading to a submaximal relaxant effect of 81.4 ± 5.81% (Calzetta et al., 2017a).

The combination between the LABA olodaterol (OLO) and the LAMA tiotropium bromide (TIO) administered at low concentrations produced a strong to very strong synergistic relaxant effect in both medium bronchi and small airways pre-contracted by carbachol (CCh) (medium bronchi: OLO: 3 nM–160 nM, TIO: 9 nM–54 nM; small airways: OLO 1.5 nM–25 nM, TIO 1.5 nM–3.6 nM). The higher bronchorelaxant response was +22.13 ± 4.42% in medium bronchi and +26.31 ± 12.39% in small airways, compared to the expected additive effect (Calzetta et al., 2019a). OLO/TIO also elicited a synergistic bronchial relaxation of medium bronchi contracted by EFS_{10Hz}, that was sustained from 2 h to 9 h post-treatment, and produced a maximal increased relaxant response of +29.37 ± 7.59% compared to the expected additive effect (Calzetta et al., 2019a). When administered at low isoeffective concentrations (EC_{20}), OLO/TIO induced a maximal relaxation of 38.50 ± 11.56%, an effect that was further enhanced up to 73.60 ± 3.10% at 11 h post-administration and remained stable for 12 h (Calzetta et al., 2019a).

3.1.2.2. Interaction between β_2-AR agonists and inhaled corticosteroids. FF combined with the inhaled corticosteroid (ICS) beclomethasone dipropionate (BDP) administered at the concentration-ratio 6:100 reproducing the FDC currently available for the treatment of asthma (Dhillon and Keating, 2006), synergistically relaxed medium bronchi pre-contracted by His (Calzetta et al., 2018c). It was confirmed that at the ratio of concentrations 6:100, FF/BDP was a balanced combination inducing synergism from low to high concentrations (Calzetta et al., 2018c). The maximal synergistic bronchorelaxant effect of +28.73 ± 7.25% was achieved with FF/BDP 0.6/10 ng/mL, compared to the expected additive effect (Calzetta et al., 2018c). Synergism was already detected at low concentrations inducing ≤25% E_{max}, whereas for concentrations eliciting ≥50% E_{max} the extent of synergism was strong (Calzetta et al., 2018c).

In small airways pre-contracted by His, FF/BDP produced a synergistic bronchorelaxant effect, and when combined at 0.06/1 μg/mL, FF/BDP induced a maximal relaxation that was +20.41 ± 4.10% greater than the additive effect (Calzetta et al., 2018c). The CI approach indicated that FF/BDP 6:100 concentration-ratio, produced a greater synergistic interaction when administered at higher concentrations, thus the extent of synergism was directly related to the concentrations of drug mixture: it was strong over the range of concentrations inducing 25–50% E_{max} and very strong when administered at higher concentrations (Calzetta et al., 2018c).

In passively sensitized medium bronchi pre-contracted by His, a procedure that reproduces ex vivo the airway hyperresponsiveness (AHR) typical of asthma, FF/BDP induced a synergistic interaction that remained stable over the range of concentrations 0.06/1 ng/mL – 6/100 ng/mL, leading to a maximal synergistic bronchorelaxant effect of +12.74 ± 4.62% greater than the additive effect (Calzetta et al., 2018c). It was confirmed that at the concentration-ratio 6:100, FF/BDP was a balanced combination producing a synergistic response from low to high concentrations (Calzetta et al., 2018c).

In passively sensitized small airways pre-contracted by His, FF/BDP administered at 0.6/10 ng/mL elicited a maximal synergistic bronchorelaxant response of +20.04 ± 2.18%, compared to the additive effect (Calzetta et al., 2018c). The CI approach indicated that FF/BDP 6:100 concentration-ratio, produced a greater synergistic interaction when administered at lower concentrations, and the extent of synergism was inversely related to the concentrations of drug mixture: it was very strong over the range of concentrations inducing 15–25% E_{max} and strong for concentrations inducing 75% E_{max} (Calzetta et al., 2018c).

3.1.2.3. Interaction between muscarinic antagonists and inhaled corticosteroids. The LAMA GLY combined with the ICS BDP at low concentrations inducing EC_{30} did not synergistically interact with both non-sensitized medium bronchi and small airways pre-contracted by His (Cazzola et al., 2016a). Conversely, in passively sensitized medium bronchi and small airways pre-contracted by His, GLY/BDP induced a synergistic relaxant response +13.71 ± 1.60% and +22.30 ± 5.39% respectively, higher than the expected additive effect (Cazzola et al., 2016a). The bronchorelaxation achieved with low concentrations of GLY/BDP inducing EC_{30} was 64.71 ± 1.60% and 73.30 ± 5.39% respectively in passively-sensitized medium bronchi and small airways (Cazzola et al., 2016a).

3.1.2.4. Interaction between β_2-AR agonists and dual phosphodiesterase 3/4 inhibitors. The short-acting β_2-AR agonist (SABA) salbutamol combined with the dual phosphodiesterase (PDE) 3/4 inhibitor ensifentrine (also known as RPL554) 1 μM or 10 μM induced a weak synergistic relaxant effect in medium bronchi pre-contracted by ACh. The maximal synergistic effect was achieved by salbutamol 100 nM plus ensifentrine 1 μM (delta effect: 0.29 ± 0.11 compared to the expected relaxant response) (Calzetta et al., 2013). No synergistic interaction was detected when salbutamol and ensifentrine were combined at lower concentrations (Calzetta et al., 2013). When administered at isomolar concentrations (1:1), salbutamol plus ensifentrine elicited an overall weak synergistic interaction: the Bliss Independent analysis detected only a signal for synergism, whereas the analysis based on the concept of dose equivalence revealed no synergistic interaction (Calzetta et al., 2013).

3.1.2.5. Interaction between muscarinic antagonists and dual phosphodiesterase 3/4 inhibitors. GLY combined with ensifentrine at low concentrations (EC_{30}) synergistically relaxed medium bronchi contracted by $EFS_{3–25Hz}$, leading to the highest bronchorelaxation after 50 ± 10 min (Calzetta et al., 2015b). The maximal synergistic reduction in the EFS-induced contractile response was −71.4 ± 5.1% at 2 h post-administration and the effect lasted up to 6 h, when the bronchial tone reduced by −41.2 ± 8.5% (Calzetta et al., 2015b). In medium bronchi pre-contracted by ACh or His, GLY/ensifentrine administered at low isoeffective concentrations inducing EC_{20} (GLY: 0.7 ± 0.4 nM and ensifentrine: 6.0 ± 1.5 μM on contraction with ACh; GLY: 1.4 ± 0.5 μM and ensifentrine: 1.7 ± 0.8 μM on contraction with His) produced a synergistic relaxation (delta effect: 0.46 ± 0.03 compared to the expected bronchorelaxant response) (Calzetta et al., 2013).

The short-acting muscarinic antagonist (SAMA) atropine (ATR) plus ensifentrine administered from 1 nM to 10 μM induced a synergistic interaction in medium bronchi pre-contracted by ACh (Calzetta et al., 2013). The maximal synergism was detected when ATR 10 nM was combined with ensifentrine 1 μM (Calzetta et al., 2013). The presence of a synergistic interaction was confirmed also when ATR and ensifentrine were administered at isomolar concentrations (1:1) (Calzetta et al., 2013).

In small airways pre-contracted by CCh, GLY/ensifentrine administered at low-to-middle concentrations (EC_{30-40}) synergistically improved the bronchorelaxation by +21.05 ± 4.02% compared to the expected additive effect (Calzetta et al., 2015b). GLY plus ensifentrine administered at low isoeffective concentrations (EC_{30}) enhanced the luminal area of the small airways by 69.08 ± 2.41% compared to the additive response (Calzetta et al., 2015b). The maximal synergistic interaction was +28.04 ± 8.66% at ≃30 min post-administration, compared to the additive effect (Calzetta et al., 2015b). The bronchorelaxation induced by GLY/ensifentrine at low concentrations (EC_{30}) lasted up to 6 h post-treatment, when the luminal area was still increased by +29.30 ± 2.04% compared to the effect of the monocomponents. The E_{max} was detected after ≃1 h post-administration, with the area of the bronchial lumen improved by +65.60 ± 9.20% (Calzetta et al., 2015b).

3.1.2.6. Interaction between β_2-AR agonists and proliferator-activated receptor gamma ligands. Salbutamol combined with either proliferator-activated receptor gamma (PPARγ) ligand rosiglitazone (RGZ) or prostaglandin J_2 administered from 0.1 μM to 0.5 μM, synergistically inhibited the proliferation of ASM cells stimulated with growth factor (Fogli et al., 2013). When the drug mixture was administered at concentrations 2.3–12.4-fold lower than monocomponents, the cell growth was reduced by ≃ 50.0% (Fogli et al., 2013).

3.2. Triple combination

3.2.1. Interaction between inhaled corticosteroids, β_2-AR agonists, and muscarinic antagonists

The triple ICS/LABA/LAMA combination of BDP/FF/GLY administered at the concentration-ratio 100:6:12.5 reproducing the currently approved FDC (Rogliani et al., 2020b) synergistically relaxed passively sensitized medium bronchi and small airways pre-contracted by His (Rogliani et al., 2020a). In medium bronchi, the maximal synergistic bronchorelaxant effect was detected when BDP/FF/GLY was administered at 1/0.06/0.125 ng/mL, whereas in small airways, the maximal synergism was achieved with BDP/FF/GLY 10/0.6/1.25 ng/mL (Rogliani et al., 2020a).

BDP/FF/GLY also produced a synergistic interaction in medium bronchi and small airways collected from COPD patients and pre-

contracted by CCh, an ex vivo model of stable COPD (Rogliani et al., 2020a). In medium COPD bronchi, the maximal synergistic bronchorelaxant response achieved with BDP/FF/GLY 1/0.06/0.125 μg/mL was +51.64 ± 4.41% greater that the additive effect, whereas in small COPD airways treated with BDP/FF/GLY 3/0.18/0.375 ng/mL, the maximal synergistic response was +28.85 ± 5.01% higher than the expected additive effect (Rogliani et al., 2020a). In passively sensitized medium bronchi, the extent of synergistic interaction was constantly very strong across a range of concentrations inducing 25–90% E_{max} (Rogliani et al., 2020a). In passively sensitized small airways, BDP/FF/GLY produced a very strong synergism at concentrations inducing 25–75% E_{max} and a strong synergism for concentrations inducing 90% E_{max} (Rogliani et al., 2020a). In medium bronchi collected from COPD donors, BDP/FF/GLY produced a constantly very strong synergism when administered at concentrations eliciting 25–90% E_{max}, whereas in COPD small airways, the synergism was low at concentrations inducing 25% E_{max}, strong for concentrations inducing 50% E_{max}, and very strong for concentrations eliciting ≥75% E_{max} (Rogliani et al., 2020a).

4. Discussion

Current evidence indicates that drug combinations modulating the bronchial contractility induce a synergistic relaxant effect when the individual components are combined at isoeffective concentrations (Cazzola et al., 2014, 2015b; Calzetta et al., 2013, 2017a). Since the 1990s, it is known that combining drugs at isoeffective concentrations represents the optimal condition to achieve synergy (Berenbaum, 1988).

There are several underlying mechanisms explaining the nature of drug interactions. In recent years, pharmacological research has been directed to elucidate what causes the synergism between LABAs and LAMAs (Calzetta et al., 2015a, 2018b; Cazzola and Molimard, 2010). LABAs activate β_2-ARs that in turn increase the synthesis of cyclic adenosine monophosphate (cAMP), a key mediator of ASM relaxation. On the other hand, LAMAs inhibit M_3 muscarinic ACh receptors (mAChR) expressed on ASM cells thus reducing the release of intracellular calcium and inhibiting the activity of protein kinase C and preventing ASM contractility (Calzetta et al., 2015a).

The activation of β_2-ARs and the blockade of M_3 mAChR induce a reversible switching interaction between the Ca^{++}-activated K^+ channels and protein tyrosine kinases, both essential for the enhancement of cAMP levels in ASM and the consequent bronchorelaxation (Calzetta et al., 2017b). Notably, in postganglionic parasympathetic neurons, LABAs indirectly reduce the release of ACh by the activation of β_2-ARs, and LAMAs block ganglionic transmission by inhibiting M_1 mAChR, which are facilitatory to α7 nicotinic receptors (Calzetta et al., 2017b).

Differently from medium bronchi, small airways are characterized by a non-neuronal cholinergic control of the ASM tone, in which the epithelium plays an essential role (Cazzola et al., 2016b). In particular, the LABA/LAMA combination activates β_2-ARs and blocks M_3 mAChR expressed on bronchial epithelial cells, thus inducing an inhibitory action on non-neurogenic ACh release, an effect mediated by organic cationic transporters (Cazzola et al., 2016b). Taken together, the complex cross-talk between adrenergic and cholinergic pathways leads to the synergistic interaction between LABAs and LAMAs and supports the pharmacological rationale for combining bronchodilators with different mechanisms of action (van der Molen and Cazzola, 2012).

Interestingly, several pre-clinical studies demonstrated that the LABA/LAMA synergism is a class effect, rather than the result of specific drug combinations (Cazzola et al., 2014, 2016b; Calzetta et al., 2017a, 2019a), and it has been also proven in clinical trials conducted in COPD patients (Cazzola et al., 2015a, 2015b; Donohue et al., 2016). However, we have to highlight that the extent of interaction on FEV_1 induced by LABA/LAMA combination in small pilot clinical trials (Cazzola et al., 2015a, 2015b) was of less extent when compared with the synergistic interaction detected in pre-clinical studies. Moreover, data from Phase III randomized controlled trials reported just supra-additive but not synergistic interaction on FEV_1 when dual bronchodilation therapy was administered even to responder COPD patients (Donohue et al., 2016).

The results of two studies (Calzetta et al., 2017a; Cazzola et al., 2015a) have drawn major concerns about the nature of drug interaction detected with the currently marketed FDCs IND/GLY 150/50 μg and UMEC/VI 62.5/25 μg: the combination of single agents at the concentration-ratio of FDCs elicited mainly an additive effect, whereas the combination of drugs at low isoeffective concentrations produced a synergistic interaction. This discrepancy can be explained by imbalanced drug combination between the monocomponents (Calzetta et al., 2019b). On the contrary, it seems that further FDCs, such as FF/ACL 9/322 μg (Cazzola et al., 2015b) and OLO/TIO 5/5 μg (Calzetta et al., 2019b), were correctly balanced to elicit synergistic bronchorelaxation. Thus, despite some discrepancies between pre-clinical investigations and Phase III studies, mainly due to the intrinsic difficulties in translating data from basic science research towards human studies (Seyhan, 2019), these findings highlighted the strong need to adequately balance the doses of single components in the FDCs, in order to fulfill the concept of isoeffectiveness and favour a synergistic interaction (Calzetta et al., 2017a, 2019b).

Contrary to what is known about LABA/LAMA combinations, there is still a certain gap in understanding the intimate mechanisms leading to the synergistic interaction between LABAs and ICSs (Calzetta et al., 2018d). Certainly, ICSs increase the expression of β_2-ARs and inhibit their down-regulation in response to chronic activation at the level of ASM cells, whereas LABAs may potentiate the anti-inflammatory action of ICSs in airway structural and inflammatory cells (Pelaia et al., 2015).

A central goal of asthma treatment is to achieve an adequate drug deposition to the distal airways, which are usually reached by only one third of the dose and are considerably implicated in asthma control, severity, and risk of exacerbations (Nicolini et al., 2008; Corradi et al., 2014; Carr et al., 2017). In this regard, it is particularly important the evidence that in small airways, FF/BDP administered at 6:100 concentration-ratio elicited a very strong synergism at low concentrations (Calzetta et al., 2018d). This could explain the superior efficacy in the control of asthma achieved with FF/BDP 24/400 μg FDC delivered via extrafine-formulation, compared to non-extrafine agents administered at equipotent dose (Huchon et al., 2009). This beneficial impact was further corroborated by a pilot study demonstrating that FF/BDP not only improved the closing capacity of small airways, but also reduced bronchial hyperresponsiveness in larger airways (Scichilone et al., 2010).

Emerging clinical evidence suggests the use of LAMA plus ICS for asthma (Lipworth, 2014), and the potential pharmacological rationale supporting this combination has been only recently provided (Cazzola et al., 2016a). The acute administration of GLY plus BDP induced a synergistic bronchorelaxant effect in an ex vivo model of bronchial asthma (Cazzola et al., 2016a). The synergistic interaction between GLY and BDP might be primarily explained by an increase in cAMP synthesis, which was considerably greater compared to non-sensitized airways (Cazzola et al., 2016a). GLY might have suppressed the activation of M_3 mAChR elicited by the release of endogenous ACh following passive sensitization (Ichinose et al., 1996), whereas the rapid non-genomic effects of BDP might have improved the response to $G_{s\alpha}$ stimulation in ASM (Brichetto et al., 2003). Both effects play a key role when considered that passive sensitization of airways enhances the M_2 mAChR/G_i coupled expression, phosphorylates the G_s protein, with consequent decrease in cAMP synthesis, and impairs ASM relaxation (Song et al., 2000; Hakonarson et al., 1995).

Triple ICS/LABA/LAMA combination BUD/FF/GLY administered at 100:6:12.5 concentration-ratio elicited a very strong synergistic bronchorelaxant effect in both medium bronchi and small airways (Rogliani et al., 2020a). This synergistic interaction is the result of a complex cross-talk between different pharmacological pathways, which eventually converge into the common cAMP-dependent protein kinase A pathway to induce a synergistic bronchorelaxation (Rogliani et al., 2020a). Interestingly, it was demonstrated that synergy was prevalently

associated with the activity of interacellular glucocorticoid receptors, and partially related with the activation of the G_s protein as part of the β_2 signal transduction pathway (Rogliani et al., 2020a).

The continued search for new drug classes and combinations with a better safety profile for patients with asthma or COPD has led to the development of the promising treatment with ensifentrine (Calzetta et al., 2015b). Interestingly, combining ensifentrine with GLY or atropine induced a synergistic relaxation of ASM (Calzetta et al., 2013, 2015b), which was surprisingly long-lasting when ensifentrine was combined with GLY (Calzetta et al., 2015b). On the contrary, there was no evidence of synergy with ensifentrine plus salbutamol combination, even when the monocomponents were combined at isoeffective concentrations (Calzetta et al., 2015b). The pharmacological rationale for combining ensifentrine with a LAMA, rather than a LABA, has not been demonstrated yet. However, the lack of synergy between ensifentrine and salbutamol can be explained by considering that two different drugs may elicit synergistic interaction only when they act on distinct pharmacological pathways (Cazzola et al., 2016b). In this respect, ensifentrine increases cAMP levels as it is a dual inhibitor of the PDE3/4, which are downstream effector proteins of the sympathetic signalling pathway activated by β_2-ARs (Cazzola et al., 2019). In contrast, ensifentrine and LAMAs are focused on completely distinct signal transduction pathways, that eventually converge to elicit the ASM bronchorelaxant effect (Calzetta et al., 2015b, 2018b). Elucidating the mechanism underlying the synergistic interaction between ensifentrine and LAMAs would represent a major step forward into respiratory research.

The combination between β_2 agonists and proliferator-activated receptor gamma (PPARγ) ligands was conceived with the aim of targeting cell proliferation rather than the relaxation of ASM tone: that of airway remodelling is indeed an important pathological feature of chronic

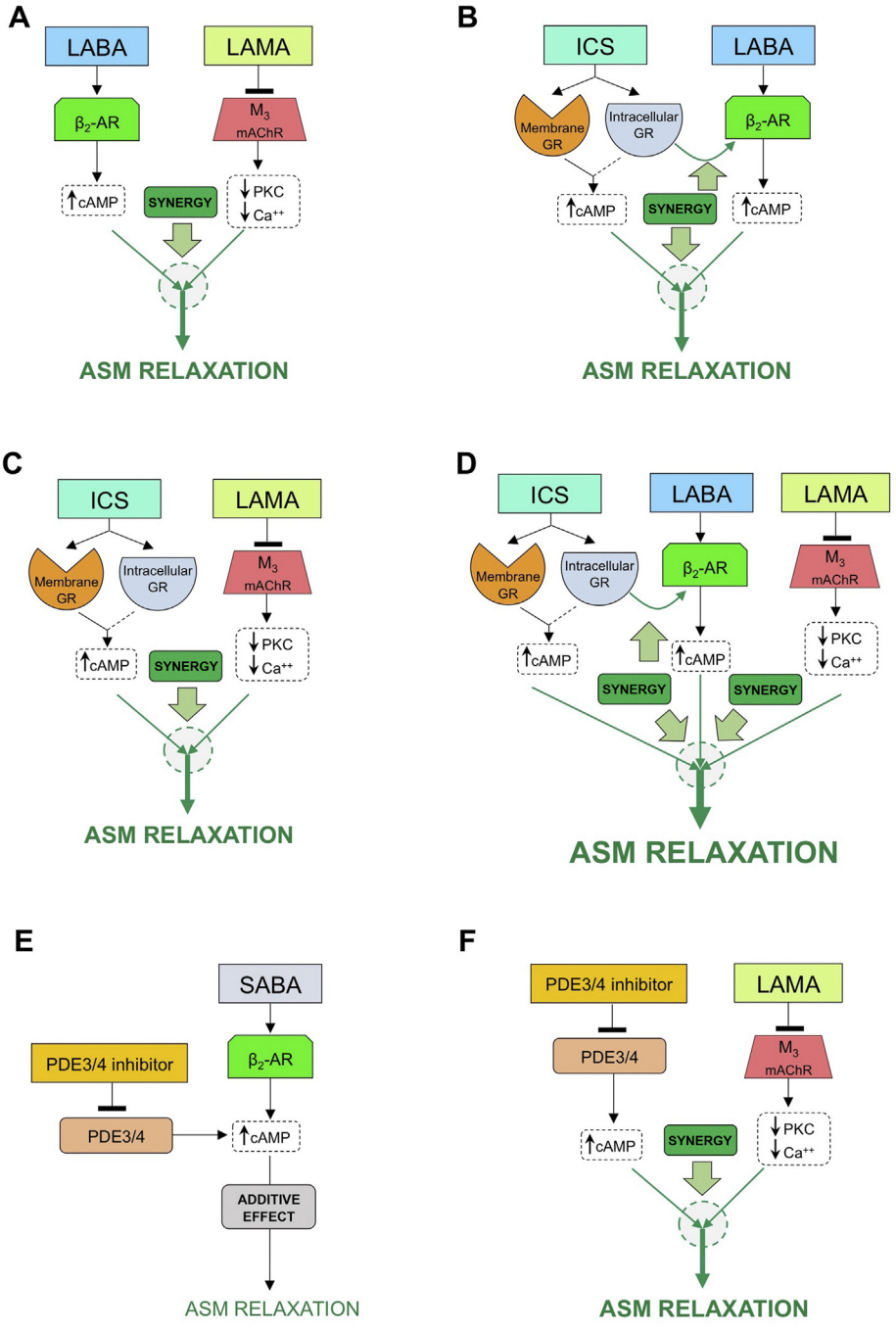

Fig. 2. Proven mechanisms of action leading to synergistic or additive interaction by combining drugs active on human airways: LABA/LAMA combination (A), ICS/LABA combination (B), ICS/LAMA combination (C), ICS/LABA/LAMA combination (D), PDE3/4 inhibitor/LABA combination (E), PDE3/4 inhibitor/LAMA combination (F). Acting on different independent signalling pathways produces a synergistic bronchorelaxant effect accordingly with the Bliss Independence model. Ca^{++}, calcium ion; cAMP, cyclic adenosine monophosphate; GR, glucocorticoid receptor; ICS, inhaled corticosteroid; LABA, long-acting β_2-adrenoceptor agonist; LAMA, long-acting muscarinic antagonist; mAChR, muscarinic acetylcholine receptor; PDE3/4, phosphodiesterase 3 and 4; PKC, protein kinase C; SABA: short-acting β_2-adrenoceptor agonist.

asthma (Fogli et al., 2013; Hough et al., 2020). Of note, the combination between PPARγ ligands and salbutamol synergistically reduced ASM proliferation and was devoid of any cytotoxic effect (Fogli et al., 2013). Such a pharmacological property suggests that the drug combination might be effective in reducing ASM hyperplasia, without affecting tissue integrity, and represents an important feature especially for asthmatic or COPD patients characterized by a rapid turnover rate of ASM cells (Fogli et al., 2013; Barnes, 2009). The molecular mechanisms underlying this synergistic interaction remain to be clarified, but it was hypothesized that PPARγ ligands preserve the antiproliferative action of β_2 agonists by reverting the desensitization of β_2-ARs following prolonged exposure to β_2 agonists (Fogli et al., 2011). Nevertheless, this effect was observed only when drugs were administered at concentration 20-fold higher than those used in the combination, thus it is unlikely to be the primary mechanism responsible of synergism (Fogli et al., 2011, 2013).

A summary of the proven interaction mechanisms leading to synergistic or additive bronchorelaxant effect in human airways are shown in Fig. 2.

5. Conclusions

The synergistic interaction elicited by drugs combined for the treatment of chronic respiratory disorders is an effect of class and it is not specific for the single drug combinations. In particular, synergy can be achieved when the combined agents are characterized by different mechanisms of action and work through distinct signalling pathways that may cross-talk with each other. Indeed, optimal synergy can be achieved only when the single agents are combined at isoeffective concentrations. This concept implies that monocomponents should be given concurrently and reach the same level of the bronchial tree in order to produce significant and durable synergistic bronchorelaxant response.

It is necessary to conduct dose-finding clinical trials specifically designed to establish the optimal concentration-ratio of the combined agents, thus allowing to identify the minimal dose for each drug to induce an appreciable synergistic interaction and optimize bronchodilation.

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CRediT authorship contribution statement

Paola Rogliani: Conceptualization, Methodology, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Funding acquisition. **Beatrice Ludovica Ritondo:** Methodology, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Bartolomeo Zerillo:** Data curation, Writing - original draft, Writing - original draft. **Maria Gabriella Matera:** Writing - original draft, Writing - review & editing. **Luigino Calzetta:** Conceptualization, Methodology, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

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References

Barnes, P.J., 2009. Chapter 51-Corticosteroids, Barnes, PJ, Drazen, JM, Rennard, SI, Thomson, NC, Asthma and COPD. Academic Press, Oxford.

Berenbaum, M.C., 1988. Isobolographic, algebraic, and search methods in the analysis of multiagent synergy. J. Am. Coll. Toxicol. 7, 927–938.

Brichetto, L., Milanese, M., Song, P., Patrone, M., Crimi, E., Rehder, K., et al., 2003. Beclomethasone rapidly ablates allergen-induced beta 2-adrenoceptor pathway dysfunction in human isolated bronchi. Am. J. Physiol. Lung Cell Mol. Physiol. 284, L133–L139.

Calzetta, L., Page, C.P., Spina, D., Cazzola, M., Rogliani, P., Facciolo, F., et al., 2013. Effect of the mixed phosphodiesterase 3/4 inhibitor RPL554 on human isolated bronchial smooth muscle tone. J. Pharmacol. Exp. Therapeut. 346, 414–423.

Calzetta, L., Matera, M.G., Cazzola, M., 2015a. Pharmacological interaction between LABAs and LAMAs in the airways: optimizing synergy. Eur. J. Pharmacol. 761, 168–173.

Calzetta, L., Cazzola, M., Page, C.P., Rogliani, P., Facciolo, F., Matera, M.G., 2015b. Pharmacological characterization of the interaction between the dual phosphodiesterase (PDE) 3/4 inhibitor RPL554 and glycopyrronium on human isolated bronchi and small airways. Pulm. Pharmacol. Therapeut. 32, 15–23.

Calzetta, L., Rogliani, P., Facciolo, F., Rendina, E., Cazzola, M., Matera, M.G., 2017a. Pharmacological characterization of the interaction between umeclidinium and vilanterol in human bronchi. Eur. J. Pharmacol. 812, 147–154.

Calzetta, L., Rogliani, P., Cavalli, F., Puxeddu, E., Facciolo, F., Cazzola, M., et al., 2017b. Protein tyrosin kinase and KCa++ cannel: two faces of the same coin in LABA/LAMA synergy. Eur Respiratory Soc 50 (61: OA4407). https://doi.org/10.1183/1393003.congress-2017.OA4407.

Calzetta, L., Matera, M.G., Rogliani, P., Cazzola, M., 2018a. Dual LABA/LAMA bronchodilators in chronic obstructive pulmonary disease: why, when, and how. Expet Rev. Respir. Med. 12, 261–264.

Calzetta, L., Matera, M.G., Cazzola, M., 2018b. Pharmacological mechanisms leading to synergy in fixed-dose dual bronchodilator therapy. Curr. Opin. Pharmacol. 40, 95–103.

Calzetta, L., Matera, M.G., Facciolo, F., Cazzola, M., Rogliani, P., 2018c. Beclomethasone dipropionate and formoterol fumarate synergistically interact in hyperresponsive medium bronchi and small airways, 19, 65.

Calzetta, L., Matera, M.G., Facciolo, F., Cazzola, M., Rogliani, P., 2018d. Beclomethasone dipropionate and formoterol fumarate synergistically interact in hyperresponsive medium bronchi and small airways. Respir. Res. 19, 65.

Calzetta, L., Rogliani, P., Page, C., Rinaldi, B., Cazzola, M., Matera, M.G., 2019a. Pharmacological characterization of the interaction between tiotropium bromide and olodaterol on human bronchi and small airways. Pulm. Pharmacol. Therapeut. 56, 39–50.

Calzetta, L., Matera, M.G., Cazzola, M., Rogliani, P., 2019b. Optimizing the development strategy of combination therapy in respiratory medicine: from isolated airways to patients. Adv. Ther. 36, 3291–3298.

Carr, T.F., Altisheh, R., Zitt, M., 2017. Small airways disease and severe asthma. World Allergy Organ. J. 10, 20.

Cazzola, M., Molimard, M., 2010. The scientific rationale for combining long-acting β2-agonists and muscarinic antagonists in COPD. Pulm. Pharmacol. Therapeut. 23, 257–267.

Cazzola, M., Calzetta, L., Page, C.P., Rogliani, P., Facciolo, F., Gavaldà, A., et al., 2014. Pharmacological characterization of the interaction between aclidinium bromide and formoterol fumarate on human isolated bronchi. Eur. J. Pharmacol. 745, 135–143.

Cazzola, M., Calzetta, L., Segreti, A., Facciolo, F., Rogliani, P., Matera, M.G., 2015a. Translational study searching for synergy between glycopyrronium and indacaterol. COPD 12, 175–181.

Cazzola, M., Calzetta, L., Ora, J., Puxeddu, E., Rogliani, P., Matera, M.G., 2015b. Searching for the synergistic effect between aclidinium and formoterol: from bench to bedside. Respir. Med. 109, 1305–1311.

Cazzola, M., Calzetta, L., Rogliani, P., Puxeddu, E., Facciolo, F., Matera, M.G., 2016a. Interaction between corticosteroids and muscarinic antagonists in human airways. Pulm. Pharmacol. Therapeut. 36, 1–9.

Cazzola, M., Calzetta, L., Puxeddu, E., Ora, J., Facciolo, F., Rogliani, P., et al., 2016b. Pharmacological characterisation of the interaction between glycopyrronium bromide and indacaterol fumarate in human isolated bronchi, small airways and bronchial epithelial cells. Respir. Res. 17, 70.

Cazzola, M., Rogliani, P., Matera, M.G., 2019. The future of bronchodilation: looking for new classes of bronchodilators. Eur. Respir. Rev. 28, 190095.

Chou, T.C., 2006. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol. Rev. 58, 621–681.
Corradi, M., Chrystyn, H., Cosio, B.G., Pirozynski, M., Loukides, S., Louis, R., et al., 2014. NEXThaler, an innovative dry powder inhaler delivering an extrafine fixed combination of beclometasone and formoterol to treat large and small airways in asthma. Expet Opin. Drug Deliv. 11, 1497–1506.
Dhillon, S., Keating, G.M., 2006. Beclometasone dipropionate/formoterol: in an HFA-propelled pressurised metered-dose inhaler. Drugs 66, 1475–1483 discussion 84-5.
Donohue, J.F., Singh, D., Munzu, C., Kilbride, S., Church, A., 2016. Magnitude of umeclidinium/vilanterol lung function effect depends on monotherapy responses: results from two randomised controlled trials. Respir. Med. 112, 65–74.
Fogli, S., Pellegrini, S., Adinolfi, B., Mariotti, V., Melissari, E., Betti, L., et al., 2011. Rosiglitazone reverses salbutamol-induced β2-adrenoceptor tolerance in airway smooth muscle. Br. J. Pharmacol. 162, 378–391.
Fogli, S., Stefanelli, F., Picchianti, L., Del Re, M., Mey, V., Bardelli, C., et al., 2013. Synergistic interaction between PPAR ligands and salbutamol on human bronchial smooth muscle cell proliferation. Br. J. Pharmacol. 168, 266–275.
Hakonarson, H., Herrick, D.J., Grunstein, M.M., 1995. Mechanism of impaired beta-adrenoceptor responsiveness in atopic sensitized airway smooth muscle. Am. J. Physiol. Lung Cell Mol. Physiol. 269, L645–L652.
Hough, K.P., Curtiss, M.L., Blain, T.J., Liu, R.M., Trevor, J., Deshane, J.S., et al., 2020. Airway remodeling in asthma. Front. Med. 7, 191.
Huchon, G., Magnussen, H., Chuchalin, A., Dymek, L., Gonod, F.B., Bousquet, J., 2009. Lung function and asthma control with beclomethasone and formoterol in a single inhaler. Respir. Med. 103, 41–49.
Ichinose, M., Miura, M., Tomaki, M., Oyake, T., Kageyama, N., Ikarashi, Y., et al., 1996. Incubation with IgE increases cholinergic neurotransmission in human airways in vitro. Am. J. Respir. Crit. Care Med. 154, 1272–1276.
Lipworth, B.J., 2014. Emerging role of long acting muscarinic antagonists for asthma. Br. J. Clin. Pharmacol. 77, 55–62.
Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., et al., 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst. Rev. 4, 1.
Nicolini, G., Scichilone, N., Bizzi, A., Papi, A., Fabbri, L.M., 2008. Beclomethasone/formoterol fixed combination for the management of asthma: patient considerations. Therapeut. Clin. Risk Manag. 4, 855–864.
Pedder, H., Sarri, G., Keeney, E., Nunes, V., Dias, S., 2016. Data extraction for complex meta-analysis (DECiMAL) guide. Syst. Rev. 5, 212.
Pelaia, G., Muzzio, C.C., Vatrella, A., Maselli, R., Magnoni, M.S., Rizzi, A., 2015. Pharmacological basis and scientific rationale underlying the targeted use of inhaled corticosteroid/long-acting β2-adrenergic agonist combinations in chronic obstructive pulmonary disease treatment. Expet Opin. Pharmacother. 16, 2009–2021.
Quirce, S., Domínguez-Ortega, J., Barranco, P., 2015. Anticholinergics for treatment of asthma. J Investig. Allergol. Clin. Immunol. 25, 84–93 quiz 4-5.
Rogliani, P., Matera, M.G., Facciolo, F., Page, C., Cazzola, M., Calzetta, L., 2020a. Beclomethasone dipropionate, formoterol fumarate and glycopyrronium bromide: synergy of triple combination therapy on human airway smooth muscle ex vivo, 177, 1150–1163.
Rogliani, P., Matera, M.G., Facciolo, F., Page, C., Cazzola, M., Calzetta, L., 2020b. Beclomethasone dipropionate, formoterol fumarate and glycopyrronium bromide: synergy of triple combination therapy on human airway smooth muscle ex vivo. Br. J. Pharmacol. 177, 1150–1163.
Schardt, C., Adams, M.B., Owens, T., Keitz, S., Fontelo, P., 2007. Utilization of the PICO framework to improve searching PubMed for clinical questions. BMC Med. Inf. Decis. Making 7, 16.
Scichilone, N., Battaglia, S., Sorino, C., Paglino, G., Martino, L., Paterno, A., et al., 2010. Effects of extra-fine inhaled beclomethasone/formoterol on both large and small airways in asthma. Allergy 65, 897–902.
Seyhan, A.A., 2019. Lost in translation: the valley of death across preclinical and clinical divide–identification of problems and overcoming obstacles. Transl. Med. Commun. 4, 1–19.
Song, P., Milanese, M., Crimi, E., Bruzzone, S., Zocchi, E., Rehder, K., et al., 2000. Gs protein dysfunction in allergen-challenged human isolated passively sensitized bronchi. Am. J. Physiol. Lung Cell Mol. Physiol. 279, L209–L215.
Us Food and Drug Administration, 2016. ANORO® ELLIPTA® Safely and Effectively. Reference ID: 3891924.
van der Molen, T., Cazzola, M., 2012. Beyond lung function in COPD management: effectiveness of LABA/LAMA combination therapy on patient-centred outcomes. Prim. Care Respir. J. : J. General Practice Airways Group 21, 101–108.