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Formoterol and beclomethasone dipropionate interact positively in antagonising bronchoconstriction and inflammation in the lung

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Abstract

These studies were designed to assess the pharmacodynamic interaction between formoterol and beclomethasone dipropionate (BDP) in controlling the bronchoconstriction and inflammatory response induced by various challenges in guinea-pigs and rats. In anaesthetised guinea-pigs, superfusion of the formoterol/BDP combination into the tracheal lumen had significantly more effect than the single components in antagonising the bronchoconstricting and inflammatory responses to acetylcholine or ovalbumin in a standard model of airway hyper-responsiveness. After ovalbumin challenge, the combination completely protected animals from death at doses lower than those effective when given separately. The combination, at doses ineffective individually, even counteracted the development of lung oedema induced by sephadex in the rat. Finally, in tracheal strips from ovalbumin-sensitised guinea-pigs pre-treatment with BDP (30 mg kg^{-1} i.m.) completely reversed the rightward shift of the formoterol dose-response curve due to β_2-receptor desensitisation. In conclusion, these results indicate that formoterol and BDP together induce a favourable pharmacodynamic interaction which can be considered more than additive, at least in these experimental settings.

Keywords: Beclomethasone dipropionate; Formoterol; Combined treatment; Bronchodilation; Anti-inflammatory activity

1. Introduction

Asthma is a chronic disease causing airway inflammation and hyper-reactivity [1]. Glucocorticoids and long-acting β_2-agonists (LABA) in combinations are widely used in its treatment [2–6]. Glucocorticoids suppress inflammation in asthma, like in other inflammatory diseases, and reduce airway hyperresponsiveness [7,8], while LABA have bronchodilatory action. They can also inhibit mast cell mediator release and plasma exudation, reduce sensory nerve activation and boost mucociliary clearance by stimulating the ciliary beat [9,10]. Thus these two classes of drugs tackle complementary aspects of the pathophysiology of asthma that neither can overcome alone. At molecular level, glucocorticoids inhibit inflammation through direct and indirect genomic effects and non-genomic mechanisms, which lead to switching off multiple inflammatory genes and favouring transcription of anti-inflammatory genes [11,7]. LABA bind to the G-protein-coupled β_2-adrenoceptor in the cell membrane, triggering a cAMP/protein kinase signalling cascade, which results in smooth muscle relaxation, and possibly anti-inflammatory effects [9].

There is a mutually favourable interaction between glucocorticoids and LABA [12–17]. Steroids accelerate the rate of transcription of the β_2-receptor gene and may also increase the efficiency of receptor coupling, thus mitigating the risk of inflammation-induced down-regulation and uncoupling; LABA increase the rate of glucocorticoid receptor translocation with additive and sometimes synergistic suppression of inflammatory mediator release [12,13].

Formoterol and beclomethasone dipropionate (BDP) are well-established representatives of the LABA and steroid classes, and offer valuable therapeutic options for treatment of asthmatic patients [18,19]. To verify the potential of a fixed combination of formoterol and BDP, a series of experimental studies was designed. Bronchodilating effectiveness was evaluated in anaesthetised guinea-pigs, with bronchoconstriction induced by acetylcholine or by lung immunological response, and anti-inflammatory efficacy in the sephadex-induced lung

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oedema model in the rat. We also verified whether, in conditions of β_2-receptor desensitisation, BDP restores the effectiveness of formoterol.

2. Materials and methods

2.1. Animals

Male Dunkin Hartley guinea-pigs (380–420 g) and male CD rats (200–250 g) supplied by Charles River Laboratories (Calco, Lecco, Italy) were used. The animals were housed in a conditioned environment ($22 \pm 1\,^{\circ}C$, $55 \pm 5\%$ relative humidity, 12-h light/12-h dark cycle) and were given free access to food and tap water. The investigation conformed with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). A separate group of guinea-pigs was actively sensitized with ovalbumin ($100\,mg\,kg^{-1}$ intraperitoneally + $100\,mg\,kg^{-1}$ subcutaneously) 21 days before use, according to the procedure described by Piper and Vane [20].

2.2. Bronchodilating activity

Guinea-pigs were anaesthetised with ethylurethane ($1.2–1.5\,g\,kg^{-1}$ i.p.) and prepared for simultaneous recording of intratracheal pressure (ITP) and systemic blood pressure (BP), according to the method already described [21]. Briefly, the trachea was cannulated for mechanical ventilation by a pump operating on a partially closed circuit ($10\,ml\,kg^{-1}$ stroke volume; $70\,cycles\,min^{-1}$). To avoid spontaneous breathing, the animals were treated with pancuronium bromide ($2\,mg\,kg^{-1}$) injected into the jugular vein. BP was monitored through the left carotid artery. All changes in ITP ($cm\,H_2O$) and BP (mmHg) were measured by pressure transducers (HP-270 and HP-1280, Hewlett Packard, Waltham, MA, USA) and the signals were displayed on a Hewlett Packard multiple-channel pen recorder (HP-7754A). Bronchoconstriction (ITP changes) was induced in normal animals by intravenous (i.v.) acetylcholine ($10–40\,\mu g\,kg^{-1}$) or ovalbumin ($5\,mg\,kg^{-1}$ i.v.) in actively sensitised guinea-pigs. Formoterol (1–300 pmol) and BDP (30–10,000 nmol) were administered alone or as a combination by superfusing the tracheal mucosa for 5 min just before challenge [22,23]. Briefly, guinea-pigs were placed in a 45° position (head up), and a thin catheter (PE-10) was introduced through the mouth into the tracheal lumen about 0.5–1 cm below the larynx. The catheter was connected to a microdialysis pump (SP-101i; 2Biological Instruments, Besozzo, Varese, Italy) for 5-min mucosal superfusion at a constant flow rate of $0.01\,ml\,min^{-1}$ (total volume, 0.05 ml). Control guinea-pigs were superfused intratracheally with saline. In some experiments BDP was superfused into the tracheal lumen for 5 min, 60 min before challenge and formoterol was given just in the last 5 min. Each animal received only one dose of formoterol and/or BDP.

Ovalbumin-sensitised animals were observed for up to 60 min after challenge to check the mortality rate, and blood samples (0.5 ml) were collected from the carotid artery at the peak of bronchoconstriction to measure circulating levels of thromboxane B_2 (TXB_2), the stable metabolite of thromboxane A_2. A commercially available specific enzyme-immunoassay kit with the following characteristics was used: minimal detectable concentration $3.6\,pg\,ml^{-1}$, 60% cross reactivity with 2,3-dinor-TXB_2 and <1% with other known prostaglandins and prostaglandin metabolites.

2.3. Sephadex-induced lung oedema in the rat

According to the method described by Belvisi et al. [24], rats were dosed intratracheally (i.t.) with vehicle (distilled water) or sephadex beads ($5\,mg\,kg^{-1}$) in a volume of $1\,ml\,kg^{-1}$ under isoflurane anaesthesia (4% in oxygen for 3 min). Treatments were administered into the tracheal lumen ($1\,ml\,kg^{-1}$) together with the sephadex. Rats were killed 24 h after the sephadex by bleeding from the abdominal aorta under isoflurane anaesthesia, and the heart and lungs were removed *en bloc*. The lung weights were measured and expressed per 100 g of body weight.

Preliminary experiments were done to determine the dose-response curves of formoterol and BDP. On the basis of these results, poorly effective doses of formoterol and BDP were chosen for further experiments in which animals were allocated to the following five treatment groups: (1) control, (2) sephadex, (3) sephadex + formoterol $0.001\,nmol\,kg^{-1}$, (4) sephadex + BDP $150\,nmol\,kg^{-1}$ and (5) sephadex + formoterol $0.001\,nmol\,kg^{-1}$ + BDP $150\,nmol\,kg^{-1}$.

2.4. β_2-Adrenoceptor-desensitised preparations

Ovalbumin-sensitised guinea pigs were killed by stunning and bleeding. In the experiments examining the effects of combined treatment, animals were given BDP ($30\,mg\,kg^{-1}$ i.m.) 24 and 1.5 h before death. The trachea was removed, cleaned and cut into two zigzag strips, according to Emmerson et al. [25]. Tracheal preparations were suspended in a 20-ml organ bath containing Krebs' solution (118 mM NaCl, 25 mM $NaHCO_3$, 4.75 mM KCl, 1.19 mM KH_2PO_4, 1.19 mM $MgSO_4$, 10 mM glucose, 2.54 mM $CaCl_2$, 0.02% ascorbic acid) maintained at 37 °C and aerated with 95% O_2 and 5% CO_2. Each preparation was subjected to 1 g of initial basal tension. Isometric tension was recorded with a force displacement transducer connected with the PowerLab system (ADInstruments, Castle Hill, NSW, Australia).

Preparations were allowed to equilibrate with frequent washing for 1 h. A sub-maximal concentration of carbachol (3×10^{-7} M) was then added to the organ bath and when stable contraction was reached, a cumulative concentration–response curve to formoterol was generated. Maximal relaxation was tested by adding a maximal dose of isoproterenol (3×10^{-7} M) at the end of the curve. Data were expressed as percentages of the isoproterenol maximal relaxation. The two strips from the same trachea were used to test formoterol with and without β_2-adrenoceptor desensitisation. One strip was left untreated, and the other was subjected to two 20-min incubation periods with a supra-maximal concentration of salbutamol (5×10^{-6} M), according to the procedure described by Daffonchio et al. [26].