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Effects of low- *versus* high-dose fluticasone propionate/formoterol fumarate combination therapy on AMP challenge in asthmatic patients: A double-blind, randomised clinical trial

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ABSTRACT

Background: The dose-response relationship between two dose levels of fluticasone/formoterol (*flutiform*®, 100/10 μg and 500/20 μg) was evaluated in asthmatic patients. Non-invasive inflammatory markers were used including adenosine monophosphate (AMP) challenge (primary endpoint), and sputum eosinophils and fractional exhaled nitric oxide (FeNO) (secondary endpoints).
Methods: Patients aged ≥18 years with forced expiratory volume in 1 s (FEV_1) ≥60% predicted and who required a dose of <60 mg AMP to elicit a 20% drop in FEV_1 (AMP PD_{20}) were randomised in this incomplete block, crossover study to receive 2 of 3 treatments b.i.d.: fluticasone/formoterol 500/20 μg (high dose), 100/10 μg (low dose) or placebo, during 2 periods of 28 ± 3 days each, separated by 2–3 weeks. AMP challenges were performed pre-dose and 12 h after last dose at the end of each treatment period. A series of *post hoc* analyses were performed only in patients allocated to *both* fluticasone/formoterol doses, who completed the study and had evaluable AMP PD_{20} data for both treatments ("fluticasone/formoterol subgroup"). Changes in AMP PD_{20} FEV_1, percentage sputum eosinophils and FeNO levels (Day 1 vs Day 28) between treatments were compared by an analysis of covariance (ANCOVA).
Results: Sixty-two patients were randomised and 46 completed the study. Fifteen patients received both high- and low-dose fluticasone/formoterol (*post hoc* subgroup). The difference in AMP PD_{20} for the overall population was not statistically significant between high- and low-dose fluticasone/formoterol (LS mean fold difference: 1.3; $p = 0.489$), although both dose levels were superior to placebo: high-dose vs placebo LS mean fold difference: 4.4, $p < 0.001$; low-dose vs placebo LS mean fold difference: 3.5, $p < 0.001$. In the *post hoc* subgroup, the difference in AMP PD_{20} between the doses was statistically significant in favour of the high-dose (LS mean fold difference: 2.4, $p = 0.012$). Other inflammatory parameters (sputum eosinophil counts and FeNO) showed small differences and statistically non-significant changes between high- and low-dose fluticasone/formoterol.
Conclusions: A significant dose-response was found between low- and high-dose fluticasone/formoterol in the *post hoc* subgroup (patients who received both doses), but not in the overall population, with the higher dose demonstrating a greater reduction in airway responsiveness to AMP.

1. Introduction

Effective long-term control of persistent asthma is frequently achieved with an inhaled corticosteroid (ICS) in combination with a long-acting β_2-agonist (LABA). Significant reductions in exacerbations with ICS and LABA combinations versus higher dosages of ICS

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or combinations of an ICS with other therapeutic agents have been demonstrated in clinical studies [1]. These findings have driven the development of several ICS/LABA combination inhalers.

The inhaled combination therapy *flutiform*® contains the ICS, fluticasone propionate (fluticasone), and the LABA, formoterol fumarate (formoterol). Both constituent components have favourable pharmacological and mechanistic properties compared to others in their respective classes. Fluticasone is highly lipophilic hence exhibits prolonged contact with the airway epithelium [2,3] and tissue retention compared to more hydrophilic ICSs [4,5]. It is also resistant to CYP 3A5-mediated intra-pulmonary degradation [6] (which has been implicated in corticosteroid resistance) unlike beclometasone and budesonide [7,8]. Formoterol (unlike salmeterol) has been shown to reverse ICS insensitivity under oxidative stress [9], disposal of formoterol (but not salmeterol) from smooth muscle cells is inhibited by ICS (thereby increasing local formoterol concentrations) [10], and cytokine-induced inhibition of formoterol (but not salmeterol) is completely reversed by ICS co-administration [11].

Fluticasone/formoterol combination therapy has been developed in 3 dosage strengths (50/5 μg, 125/5 μg and 250/10 μg per actuation), based on the doses approved for other fluticasone- and formoterol-containing products. Nonetheless only limited dose-response data in asthma are available for fluticasone/formoterol [12] or for other ICS/LABA combinations [13–18]. This is related to the shallow dose-response that exists for conventional clinical endpoints, particularly spirometric outcomes, routinely employed in dose-finding and pivotal registration studies. It remains important therefore to investigate a dose-response relationship to support the rationale for dose escalation.

Compared to spirometric endpoints, airway hyperresponsiveness (AHR) to adenosine 5'-monophophate (AMP) [19], is a sensitive marker for defining dose-response for ICSs or ICS/LABA combinations [20–23]. Other ICS-sensitive inflammatory biomarkers include fractional exhaled nitric oxide (FeNO) [24–27] and sputum eosinophils [26,28] which have also been shown to exhibit a dose-response [24–28].

This is only one of two studies that aimed to evaluate the dose-response relationship between two dose levels of fluticasone/formoterol (100/10 μg b.i.d. and 500/20 μg b.i.d.) and the first on non-invasive inflammatory markers including AMP challenge (primary endpoint) and sputum eosinophils and FeNO (secondary endpoints) in asthmatic patients.

2. Methods

2.1. Participants

Eligible patients were ≥18 years, non-/ex-smokers (<10 pack years), with a pre-bronchodilator forced expiratory volume in 1 s (FEV_1) ≥ 60% predicted and required a dose of <60 mg AMP to elicit a 20% drop in FEV_1 (AMP PD_{20}). Key exclusion criteria included: other clinically significant respiratory or cardiac diseases, medications considered likely to interfere with the study outcomes, hospitalisation or ER attendance or respiratory tract infections within 4 weeks of screening. The following medications were not allowed during study and needed to be discontinued before screening: omalizumab (6 months), systemic corticosteroids (12 weeks), antihistamines (2 weeks), cromones and leukotriene receptor antagonists (1 week). Patients were recruited at 7 sites in Germany. The study was performed in accordance with the Declaration of Helsinki, the ICH Harmonised Tripartite Guidelines for Good Clinical Practice (GCP), and with applicable regulatory requirements. The study protocol was reviewed by the central ethics committee (Schleswig Holstein). All patients gave written informed consent prior to screening. EudraCT Number: 2009-009873-87; clinicaltrials.gov identifier: NCT00995800.

2.2. Study design

This was a multi-centre, double-blind, randomised, incomplete block, crossover study evaluating the effects of two dose levels of fluticasone/formoterol (100/10 μg b.i.d. and 500/20 μg b.i.d.) and placebo on AHR to AMP and other markers of airway inflammation (Fig. 1).

The study consisted of an initial 14–21 day wash-out period, followed by two double-blind 28-day treatment periods separated by a second 14–21 day wash-out period. At the start and end of each treatment period, FeNO measurement was followed by spirometry, AMP challenge and sputum induction. Given the length of both wash-out and treatment periods, an incomplete block, 2-treatment period design was considered more feasible than a complete-block approach. During the wash-out periods patients took only salbutamol (Ventolin® Evohaler®) as required. Patients were contacted by telephone on Day 27 of each treatment period to ensure that the last dose of study medication was taken that evening (12 h prior to the Day 28 visit). A follow-up telephone call took place 7–10 days after last dosing.

2.3. Study treatment and dose rationale

Randomisation was performed using a validated system that assigned eligible patients to receive 2 of the 3 study treatments below in 1 of 6 sequences:

- Fluticasone/formoterol 250/10 μg 2 puffs b.i.d. (500/20 μg b.i.d.)
- Fluticasone/formoterol 50/5 μg 2 puffs b.i.d. (100/10 μg b.i.d.)
- Placebo 2 puffs b.i.d.

All treatments were administered via the AeroChamber Plus® spacer device. The doses of fluticasone/formoterol were selected as a pairwise dose-response was considered unlikely to be seen without a four- to five-fold ICS dose difference [20,27–30].

2.4. Methods

FeNO was measured with a NIOX-MINO™ analyser (Aerocrine, Solna, Sweden) per current guidelines [31]. Only one satisfactory measurement was performed [32]. Spirometry was performed in accordance with the American Thoracic Society/European Respiratory Society Guidelines (2005) [33]. Predicted values for adults were calculated according to Quanjer et al., 1993 [34]. If baseline FEV_1 was ≥60% predicted, AMP challenge (0.39–800 mg) was performed using the 5 deep breath dosimeter method, until a AMP PD_{20} was reached or the highest dose inhaled, according to a previously described protocol [35]. Pre-challenge, patients refrained from short-acting β_2-agonists (SABAs) and LABAs for at least 6 and 12 h, respectively. After each inhalation, the patient held their breath at total lung capacity for 2–3 s. After each cycle of 5 breaths, a lung function test was performed in duplicate, 2 min after the last deep breath [36]. If FEV_1 values were more than 5% apart spirometry was repeated. The best post-saline FEV_1 was used as a baseline. At the start of the second treatment period, AMP PD_{20} had to be within 1.5 doubling doses of that at the start of treatment period 1 to ensure comparability between periods. After AMP challenge, patients were given 200 μg salbutamol followed by sputum induction and processing per standardised protocols [37–39]. Sputum processing was performed by a trained nurse or physician at each site. Eosinophil counts were assessed from 400 non-squamous nucleated cells on stained (HemaColor®, Merck,