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Bioequivalency Study for Inhaled Drugs: A Pharmacodynamic Approach

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Abstract

The study was performed to evaluate the bioequivalence of two marketed dry powder inhaler products formoterol 6 mcg associated with and budesonide 200 mcg Alenia ® from Biosintética Farmacêutica Ltd. and Symbicort ®. AstraZeneca, Brazil) in 27 volunteers patients of both sexes. The study used an open, randomized, 2 period crossover balanced design, with a 36 days wash out period between the doses. The pharmacodynamic evaluation of formoterol and budesonide was performed by Spirometry, comparing the response of the two products to prevent wheezing illness (bronchial obstruction) induced by methacholine. The mean ratio of AUC_{0-t} parameters and 90% confidence intervals were calculated to determine the pharmacodynamic responses. Geometric mean for the test and reference formulation of formoterol with budesonide in the form of dry powder in capsule was AUC0-t ratio (test/reference: 101.70% [98.53% - 104.98%]).

We concluded that the formulations Alenia® and Symbicort® are therapeutically equivalent, considering the confidence intervals (90%) of the ratios between the geometric means of the test and reference formulations, the AUC_{0-t} parameter, and taking into account this is a pharmacodynamic study, in which intervals to determine therapeutic equivalence have not been established yet. The limits of the confidence interval of the parameters studied are within the range established by RE 1170 (April 2006/ANVISA) for pharmacokinetic parameters.

Keywords: Formoterol; Budesonide; Pharmacodynamics; Bioequivalence

Introduction

Combination therapy of inhaled corticosteroids (ICS) and long-acting beta2-agonists (LABA) have become standard of care for the treatment of asthma, in those not fully responsive to ICS alone, as they have been clinically proven to reduce morbidity and improve airway function [1-4]. There is increasing data that suggests a synergistic therapeutic relationship exists between the ICS and LABA [5]. Inhaled corticosteroids are by far the most effective controllers used in the treatment of asthma, and the only drugs that can effectively suppress the characteristic inflammation in asthmatic airways, even in very low doses. In contrast, ICS are largely ineffective in suppressing pulmonary inflammation in chronic obstructive pulmonary disease (COPD) and have a poor clinical effect. In both asthma, and COPD ICS are commonly given as combination inhalers with long acting β2-agonists (LABA) [6].

Formoterol fumarate is a long-acting selective beta2-adrenergic agonist (beta2-agonist) with a rapid onset of action. Inhaled formoterol fumarate acts locally in the lung as a bronchodilator. The pharmacological effects of beta2-adrenoceptor agonist drugs, including formoterol, are at least in part attributable to stimulation of intracellular adenyl cyclase, the enzyme that catalyzes the conversion of adenosine triphosphate (ATP) to cyclic-3', 5'-adenosine monophosphate (cyclic AMP). Increased cyclic AMP levels cause relaxation of bronchial smooth muscle and inhibition of release of mediators of immediate hypersensitivity from cells, especially from mast cells. Inhaled formoterol is rapidly absorbed; peak plasma concentrations are typically reached at the first plasma sampling time, within 5-10 minutes after dosing. As with many drug products for oral inhalation, it is likely that the majority of the inhaled formoterol delivered is swallowed and then absorbed from the gastrointestinal tract. Charcoal block was not considered as this was a pharmacodynamic study, with a descriptive approach of the kinetics profile obtained from the literature. It was reported that Inhaled formoterol is rapidly absorbed; peak plasma concentrations are typically reached at the first plasma sampling time, within 5-10 minutes after dosing.

The primary metabolism of formoterol is by direct glucuronidation and by O-demethylation, followed by conjugation to inactive metabolites. Secondary metabolic pathways include deformylation and sulfate conjugation. CYP2D6 and CYP2C have been identified as being primarily responsible for O-demethylation [7,8].

Budesonide is an anti-inflammatory corticosteroid that exhibits potent glucocorticoid activity and weak mineralocorticoid activity. Inflammation is an important component in the pathogenesis of asthma. Corticosteroids have a wide range of inhibitory activities against multiple cell types and mediators involved in allergic and non-allergic-mediated inflammation. These anti-inflammatory actions of corticosteroids may contribute to their efficacy in asthma. Orally inhaled budesonide is rapidly absorbed in the lungs and peak concentration is typically reached within 20 minutes. After oral administration of budesonide, peak plasma concentration was achieved in about 1 to 2 hours and the absolute systemic availability was 6-13%, due to extensive first pass metabolism. In contrast, most of the budesonide delivered to

the lungs was systemically absorbed. Budesonide was excreted in urine and feces in the form of metabolites [7,8].

Traditionally inhaled treatment for asthma has been considered as preventer and reliever therapy. The combination of formoterol and budesonide in a single inhaler introduces the possibility of using a single inhaler for both prevention and relief of symptoms. A single combination inhaler containing formoterol and budesonide has been advocated for regular maintenance of asthma, with the option to increase the dose if the asthma flares up. Single inhaler therapy can reduce the risk of asthma exacerbation.

This study aims at describing the benefits of this combination of another similar medication, in the market of similar drugs, with proved efficacy and safety when compared to the reference drug.

Methods

Study protocol

The study was performed in accordance with the Helsinki Declaration and Good Clinical Practice Guideline, and informed consent was obtained from participants prior to the onset of the study. The clinical part of the study was carried out at Scentryphar Clinical Research (Campinas City, São Paulo, Brazil).

Subjects

Twenty seven patients with mild persistent asthma, of both sexes, ages between 19 and 39 (mean ± SEM: 27.72 ± 5.95 years), between 1.52 m and 1.85 m (1.67 ± 0.11 m) high, weighing between 67.50 kg and 101.00 kg (69.54 ± 0.74 kg) and within 15% of their ideal body weight were enrolled in the study. Subjects were considered eligible for enrolment in this study if they were in compliance with all the inclusion and exclusion criteria described in the protocol.

All the subjects provided written informed consent to participate after being told about the nature and purpose of the study. The study protocol was approved by the University of Campinas/Unicamp, in compliance with the ethical principles described in the Declaration of Helsinki, guidelines for International Conference on Harmonization-Good clinical practices (ICH-GCP).

All volunteers were assessed by physical examination, ECG, and the following laboratory tests: blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, Gamma GT, total bilirrubin, albumin and total protein, triglycerides, total cholesterol, hemoglobin, hematocrit, total differential white cell counts, routine urine and spirometry (volume of air inhaled and exhaled testing). All subjects were negative for HIV, HBV (except for serological scare) and HCV.

Drug products

The test formulation employed was Alenia® powder for inhalation (Formoterol 6mcg and Budesonide 200 mcg, batch number 0700824) and Symbicort® powder for inhalation (Formoterol 6mcg and Budesonide 200 mcg, batch number IC1558).

Study design

The study was performed to evaluate the pharmacodynamic of formoterol 6mcg and budesonide 200 mcg powder formulation for inhalion (Alenia® from Biosintética Farmacêutica Ltd as test formulation, and Symbicort® from AstraZeneca as reference formulation) under fed conditions. ALENIA was tested for bioequivalence for the first time.

For this study it was used an open, randomized, 2 period crossover balanced design with a 36 days wash out period between the doses. After a supervised fast of 10 hours at least, the subjects were served a meal: plain milk (200 mL) and 01 bread roll (50 g). These meals were finished within 60 min and before drug administration, then the subjects were dosed 60 minutes after starting the meal. The meals were identical for both study periods. No other food was permitted during the confinement period. Liquid consumption was allowed *ad libitum* 2 hours after drug administration. However, xanthine-containing drinks including tea, coffee, and cola were avoided.

Clinical parameters were obtained directly by evaluating the pulmonary function test of volunteers to measure the response of the two drugs (budesonide and formoterol) together against a bronchial obstruction induced by methacholine, based - on the application of a non-compartmental model, suitable for evaluation of these responses after administering the drug orally through inhalation.

Study procedure

The evaluation of the pharmacodynamic of test and reference formulations was performed by Spirometry, comparing the process to prevent wheezing illness (bronchial obstruction) induced by methacholine.

Prior to treatment, the volunteers were hospitalized to determine the individual dose of methacholine required (substance used to induce a bronchial obstruction), ie the dose capable of promoting the reduction equal to or greater than 20% in forced expiratory volume in one second (FEV1), for the evaluation function test (spirometry).

On the morning of Day 1 of each treatment period, volunteers performed a spirometry to determine FEV 1 pre - treatment. In each period the volunteers were given for 10 days, two doses every 12 hours (twice a day) oral formulation of the test or reference formulation for aspiration, as randomization of the study. At the time of each dose, the subject performed a deep inspiration, from functional residual capacity to total lung capacity and maintained an inspiratory pause before starting the expiration. Before each administration, peak expiratory flow (PEF) was measured 3 (three) times through the Peak Flow Meter and the highest value was noted. On the night of the 10th day the volunteers were confined. On the eleventh day, the volunteers received a dose of test or reference medication, according to the schedule of treatment and underwent bronchoprovocation testing. Volunteers remain hospitalized for about 17 hours after drug administration.

First three curves were obtained to measure the FEV1, while the highest value of this FEV1 baseline was considered the reference for calculating the percentage of decrease. After that, the measure was carried out with methacholine inhalation in a concentration that led to the downfall of not less than 20% in FEV1, in the test performed prior to treatment. To assess bronchodilator FEV1 was measured 30, 60, 90 and 120 seconds after the inhaled methacholine.

The volunteers performed the spirometry of periods 1 and 2 at the same time it was performed for baseline spirometry.

To reverse the bronchoconstriction, salbutamol was administered, after which spirometry was performed for safety.

As a safety measure during the treatment period, blood was collected for cortisol.

Pulmonary function tests

The forced vital capacity (FVC) was determined by means of a spirometer with a flow sensor (SpiroBank G, MIR, Italy) connected to a computer to obtain and to analyze the data of the inspiratory and expiratory efforts (winspiroPRO, MIR, Italy). The spirometry was carried out according to American Thoracic Society (ATS) and European Respiratory Society guidelines [10].

Pharmacodynamic analysis and statistical analysis

Pharmacodynamic parameter analysed was area under the curve (AUC_{0-t}) from 0 to time t (time of the last measured FEV1 under drug effect – 120 sec), calculated by trapezoidal method.

The FEV1 means observed during selection, pre-methacholine, pre-T and pre-R phases were compared by fitting a mixed linear model using phase as fixed factor and patient as random effect.

In order to evaluate efficacy of the medication, the equivalence between the T and R formulations and FEV1 means before and after medication (30 and 90 sec) for T and R formulations had been previously analyzed. The analyses were carried out by fitting a doubly repeated measurement (period and times within periods) model. The response was FEV1, the fixed factors were formulation, pre/pos-medication, time of measurement, interaction formulation-by-time, formulation-by-pre/pos and pre/pos-by-time. Patient was used as a random effect. As suggested by residual analysis, log-natural transformation was used for response variable.

To verify the equivalence between the formulations, log-transformed AUC_{0-t} was used as the response and a linear mixed model was fitted. The fixed effects were sequence (RT e TR), period (1 e 2), formulation (R e T), and patient within sequence was the random effect.

All the analyses were carried out using the SAS® (Statistical Analysis System) version 9.3.1.

Results

Tolerability analysis

Formoterol and budesonide formulation was well tolerated at the administered dose. All the biochemical parameters did not present any clinical relevant alterations. No serious adverse effects were either reported or observed.

Pharmacodynamic and statistical analysis

The arithmetic means and confidence intervals (90%) of FEV1 in the stages of the study are presented in Figure 1.

The resulting 90% confidence intervals of the parameter ratios for AUC_{0-t} are summarized in Table 1.

Discussion

Asthma is a chronic inflammatory disease characterized by reversible airways obstruction, airways remodeling and nonspecific airways hyper-responsiveness (AHR) [11]. A multicentric study (International Study for Asthma and Allergies in Childhood - ISAAC) conducted in 56 countries showed a variation of active asthma from 1.6% to 36.8%. In Brazil, the average prevalence was 20% [12]. In 2005, the Ministry of Health reported 293,427 hospitalizations and 2,603 deaths from asthma in Brazil [13,14].
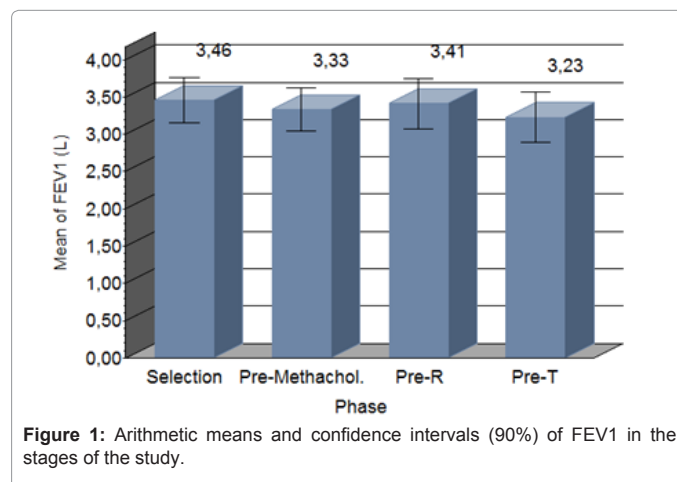

Figure 1: Arithmetic means and confidence intervals (90%) of FEV1 in the stages of the study.

Parameter	Ratio T/R (%)	Lower Limit (%)	Upper Limit (%)	Power (%)	Coefficient of Variation (%)
ASC (0-1)	101,70	98,53	104,98	99,99	6,82

Table 1: Ratios means and the 90% geometric confidence interval of test and reference formulation.

The inhaled corticosteroids are currently the most effective drugs for the control of asthma [15]. The maintenance treatment with inhaled corticosteroid reduces the frequency and severity of exacerbations, the number of hospitalizations and deaths [16-18].

In patients with severe asthma, the combination of inhaled corticosteroids in moderate doses with long acting β2-agonists results in significantly greater clinical benefit than increasing the dose of inhaled corticosteroids by twofold or more, and allows the clinical control of asthma in a larger number of patients, more quickly, than the single use of inhaled corticosteroids [19,20].

Controlled studies have shown that the joint administration of these drugs is as effective as the administration by different systems [21,22]. Moreover, the combination of budesonide and formoterol can be used in both the maintenance and rescue treatment [15]. However, it is necessary to emphasize that the increase of the benefits of new treatments to the population is closely linked to the policy of generic drugs. The benefits goes beyond the limits of individual care and reach the whole society because it reduces the impact of asthma in the economic and social cost, for example, direct medical care and lost days of work or study.

Bioequivalence studies are important in the development of inhaled medications and delivery devices. For inhaled medications, bioequivalence ensures that equal doses of different agents of the same class, or different formulations of the same medication (i.e., powdered or aerosolized), produce equivalent pharmacodynamic effects. Pharmacodynamic clinical efficacy studies are considered the most useful tool to assess bioequivalence of different inhaled medications or different inhalation devices [23,24].

The purpose of the present study was, first, to evaluate the pharmacodynamic response of two marketed dry powder inhaler products containing formoterol 6 mcg and budesonide 200 mcg (Alenia® from Biosintética Farmacêutica Ltda and Symbicort® from

AstraZeneca, Brazil), and ,second, the product safety by monitoring serum cortisol and the occurrence of Adverse Events.

The mean ratio of AUC_{0-t} parameters and 90% confidence intervals were calculated to determine the pharmacodynamic response. The point estimator and the 90% confidence intervals for the AUC_{0-t} ratio (test/reference: 101.70% [98.53% - 104.98%]) indicate high similarity of both formulations with respect to the extent of formoterol associated with budesonide. Therefore, we concluded that the formulation of Alenia® and Symbicort® are therapeutically equivalent, taking into account this is a pharmacodynamic study, in which intervals to determine therapeutic equivalence have not been established yet. The limits of the confidence interval of the parameters studied are within the range established by RE 1170 (April 2006/ANVISA) for pharmacokinetic parameters.

Acknowledgments

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