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A New Approach to Bioequivalence Studies for Nasal Sprays: A Nasal Challenge Considering Histamine Intermediate-Late Phase Reaction

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Abstract

Topical intranasal corticosteroids are widely recognized as the first-line anti-inflammatory treatment. Many of the most-prescribed nasal sprays containing local action drugs are expected to go off patent, with a consequent increase of the generic copies of these medications, creating greater product competition and consequently, price reduction. Bioequivalence studies for nasal sprays are still under discussion. The study designs for this purpose generally use long-term therapeutic intervention models with a high cost and long-term treatment of patients. This study was designed to demonstrate the feasibility of rhinomanometry in bioequivalence studies for nasal sprays. An open, randomized, crossover study, using two periods and two sequences to evaluate pharmacodynamic equivalence between two formulations of beclomethasone dipropionate spray. After nasal challenge with histamine, 25 healthy volunteers were submitted to an anterior rhinomanometry at the time 0; 15; 30 and 60 minutes building a baseline of flow, pressure and resistance of nasal chamber. The volunteers were then submitted to nasal drug spray Test (**T**) or Reference (**R**) of beclomethasone dipropionate, according to a randomized schedule. The Area Under Curve (AUC_{0-t}) was analyzed. The ratio between the geometrics averages of AUC_{0-t} from **T** and **R** was 1.08 for 90% CI (0.2451; 0.2259), suggesting the bioequivalence between formulations.

Keywords: Rhinomanometry; Histamine; Nasal Provocation Tests; Nasal Sprays; Bioequivalence Trials; Rhinitis

Introduction

Epidemiological studies have shown the increase in the prevalence of allergic rhinitis around the world suggesting a universal behaviour to conditions [1-4]. The most common symptom of allergic rhinitis is nasal blockage, often accompanied by discharge, itching and sneezing [1,5,6]. The persistence of these symptoms can affect quality and life conditions [2,5,7,8]. Histamine is one the most important components released during the allergic reaction [9-11]. When instilled locally in the nose, histamine produces a similar response of the nasal mucosa [12-14]. The mechanisms of allergic rhinitis have been clarified by using nasal challenge with allergen or pro-inflammatory mediators and by measuring cells and mediators released during the early and late-phases of an allergic reaction. However, the priming effect of the nasal mucosa as a single challenge does not perfectly mimic the ongoing allergic reactions induced by repeated allergen exposure.

The inflammation model studied in allergic rhinitis takes into account the alterations of the nasal compartment, such as edema, increase of mucous secretion, increase in vascular permeability, increase of inflammatory cells, and nasal symptoms.

Quantification of nasal stuffiness by measuring nasal airway resistance is a convenient method where objective information of changes in the intranasal conditions is required as a specific nasal challenge or evaluation of drug treatment.

The nasal provocation test is a standardized method to diagnose suspected allergies [15,16] and a useful tool in allergy research [1,17]. In this test, reactions are observed in response to potential allergens or to histamine placed in the nasal cavity [15]. Among other symptoms such as sneezing, nasal secretion, itchiness, and lacrimation, swelling of the nasal mucosa is considered an indication of inflammatory reaction. Several methods are used to measure changes in nasal congestion during nasal provocation. Anterior rhinomanometry is routinely carried out for the assessment of the effects of nasal provocation [18-20], allowing the measurement of respiratory resistance in the nasal passage, a parameter that reflects the degree of obstruction due to swelling of the mucosa or other anatomic changes [21-24].

Rhinomanometry is a well established for quantitative assessments of nasal airway respiratory function. Sensitivity, specificity and reproducibility of results obtained are well within the range of results obtained by other generally accepted laboratory procedures [25]

Topically-delivered intranasal corticosteroids (INCS) are widely recognized as a first-line anti-inflammatory treatment [26-28]. The inflammatory reaction after allergen challenge or environmental exposure increases the responsiveness to specific (allergen) and nonspecific (histamine, methacholine, cold) stimulation. Is well documented that these responses are reduced by intranasal corticosteroids [27,29]. The explanation for these properties include a reduction in the number of epithelial mast cells and an effect on sensory nerves, epithelial cells, and submucosal glands [27].The occurrence and severity of side effects with INCS depend on a large number of variables, including the drug's properties (lipophilicity, pharmacokinetics, pharmacodynamics), as well as dosage and susceptibility of the patient. In the usual dose, for example, the systemic INCS side effects of beclomethasone dipropionate (BDP), at 400mcg/

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day, do not represent a problem in adult subjects. The intranasal dosage used in allergic rhinitis is less than that usually prescribed for asthma treatment; hence, the risk of systemic adverse effects is lower with INCS than with inhaled corticosteroids [30].

Many of the most-prescribed nasal sprays for local action drugs are expected to go off patent, allowing the increase of the generic copies of these medications, creating greater product competition and consequently, price reduction. In most cases, generic products are available once the patent protections afforded to the original developer have expired. When generic products become available, the market competition often leads to substantially lower prices for both the original brand name product and the generic forms [31]. Bioequivalence studies for nasal sprays are still under discussion. Study designs for this purpose usually use long-term therapeutic intervention models with a high cost and long-term treatment of patients. Released in the nasal mucosa the histamine mimics the alterations found in atopic hypersensitivity, though lacking the promotion of inflammation, enough to modify the nasal compartment in a transitory way. Thus, the possibilities of studies of effect using anti-inflammatory drugs in the nasal compartment of healthy volunteers stimulated with histamine could minimize costs and time of study. Consequently, the histamine challenger model could have a potential application in studies seeking to compare the similarity between the effects of drugs.

The aim of this work was to elaborate a study model of nasal sprays using a nasal histamine challenger as a modifier of the nasal compartment and seeking to compare the similarity between the effects of different drugs for Bioequivalence studies using rhinomanometry evaluation.

Casuistic, Materials and Methods

The study populations consisted of 25 healthy subjects (14 female and 11 males, aged between 18 and 50 with a mean value of 30 years, body mass index between 19 and 28,5 (Dietary Guidelines Advisory Committee, 2005). Atopy was excluded by clinical history (asthma or allergic rhinitis), allergen skin prick test, and total IgE. Volunteers presenting detectable abnormalities in the nasal cavity by anterior rhinoscopy were excluded. No subjects were under previous medications.

Studies were approved by institutional review boards, and each patient gave informed consent. Studies were conducted in accordance with the Committee of Ethics in Research of Unicamp, Campinas, accredited by the National Commission of Ethics in Research (Conep) - National Council of Health/MS and the Helsinki Declaration and the Guidelines for Good Clinical Practice.

Study design

An open, randomized, crossover study, using two periods and two sequences to evaluate pharmacodynamic equivalence between two formulations (drug-test (T) and drug-reference(R)) of beclomethasone dipropionate nasal spray (GlenmarkFarmacêuticaLtda (T) and GlaxoSmithKline Brasil (R)). The 0.5mg/mL was the smallest histamine nasal dose response previously established using 0.3; 0.5 and 1 mg/mL. After nasal challenge with histamine (0.5mg/ml, both nostrils), volunteers were submitted to an anterior rhinomanometry at the time 0; 15; 30 and 60 minutes building a baseline of nasal chamber flow, pressure and resistance. Volunteers were then submitted to nasal drug spray (Test or Reference), according to a randomized schedule (Proc Plan; *Statistical Analysis System® versão 9.1.3; USA)*, 50g/nostril twice a day (200 g a day) for 7 days. After a 21 day washout period, the volunteers that received test or reference drug were changed.

Anterior rhinomanometry

Volunteers were measured for nasal resistance by anterior rhinomanometry (GM Instruments, Ashgrove, Kilwinning, UK) with on-line computerized integration of total nasal flow and pressure before and after histamine challenge and before and after test and reference drug administration. Total nasal flow was measured with patients breathing tidal volumes through a facemask with their months closed. Nasal pressure was measured by placing a pressure probe in the volunteers' nasal external valves. Flow rates were calculated at a nasal pressure of 150 Pa, according to the recommendation of the "Consensus report on acoustic rhinometry and rhinomanometry" [24]. Data were expressed as nasal resistance (Pa/mL/min) and nasal airflow as ml/min of the nasal chamber. All data are supplied automatically by Naris software (GM Instruments, Ashgrove, Kilwinning UK), Table 1. All rhinomanometry measurements were performed in room with controlled temperature and humidity.

Challenge protocol

Volunteers had baseline measurements of nasal rhinomanometry. Before the first anterior rhinomanometry the volunteers were acclimatized for at least 30 minutes. Two puffs of sterile saline solution (histamine diluting solution) from a nasal pump spray were delivered into both nostrils. At point 0 anterior rhinomanometry was performed followed by immediate histamine application of 0.5mg/ml/nostril via nasal spray and anterior rhinomanometry measurements were performed at: 15; 30 and 60 minutes. For the nasal application we used a similar spray container Table 1.

	Drug administration T or R*	Histamine challenge	Anterior rhinomanometry
Day 01			Basal
		X	00:00
			00:15
			00:30
			00:60
	X		
	X		
Day 02	X		
Day 03	X		
Day 04	X		
	X		
Day 05	X		
Day 06	X		
Day 07	X		
Day 08			00:00
		X	
			00:15
			00:30
			00:60
		Washout 21 days	

T: Drug-test; R: Drug-reference;

Table 1: Schedule of nasal challenge, rhinomanometric evaluation and drug administration.

Statistical analysis

The pharmacodynamic approach for comparing two nasal therapeutic spray formulations (test and reference) will be performed by indirect evaluation using the rhinomanometry measurements of volunteers. The Area Under Curve (AUC) was determined using nasal resistance and airflow at timepoints 0; 15; 30 and 60 minutes from histamine challenge.

The analyzed data are from an open, randomized, crossover study, using two periods and two sequences to evaluate pharmacodynamic equivalence between two formulations of beclometasone spray. One assumes that data are from a validated and calibrated process. $AUC_{0\text{-}t}$, area under the curve from time 0 to time *t* (the time of the last observed total resistance), calculated by linear trapezoidal method. Total resistances measured before and after medication were, for each formulation, compared to demonstrate that both had action upon nasal mucosa after histamine challenge. A linear mixed model with repeated measures was fitted using log-transformed total resistance as a response to the following factors: formulation, time (before and after medication) nested in formulation. Covariance structure used for the model repeated measures within subjects was first-order autoregressive. The analysis of log-transforming area under the curve from 0 to *t* was carried out using a linear mixed model. Independent variables were sequence (RT and TR), period (1 and 2), product (R and T) – fixed effects – and subject nested in sequence – random effect. Procedure Mixed of the SAS® System, released 9.1.3, was used to analyze the data. Variance components were estimated using restricted maximum likelihood (REML) technique and Satterthwaite's approximation for the denominator degrees of freedom.

For the purpose of bioequivalence analysis, ANOVA (analysis of variance) was performed using the Graph Pad Prism 5 software.

Bioequivalence between the Drug-Test and Drug-Reference was to be concluded if the 90% confidence interval for the $AUC_{0\text{-}\infty}$ ratio for the two treatments fell entirely within 80-125%. $AUC_{0\text{-}t}$ and C_{max} were analyzed and tested for bioequivalence in the same way as $AUC_{0\text{-}\infty}$.

Results and Discussion

Tolerability

Beclomethasone formulation was well tolerated at the administration dose (200 mcg/day). No adverse effects were reported or observed.

Rhinomanometry

The total nasal resistance records before and after treatment with the drug-test and drug-reference are shown in Table 2 and Table 3 respectively.

Gender	Age	Anterior Rhinomanometry - Total nasal resistance (Pa/mL/min)							
		Before Drug-Test				**After Drug-Test**			
		0	15 min	30min	60min	0	15 min	30min	60min
F	37	0,268	0.156	0.153	0.277	0.143	0.120	0.128	0.108
M	26	0.244	0.294	0.197	0.237	0.199	0.274	0.199	0.249
M	33	0.440	0.223	0.351	0.218	0.216	0.253	0.452	0.619
F	24	0.266	0.251	0.278	0.254	0.376	0.277	0.292	0.262
F	23	0.268	0.277	0.221	0.243	0.177	0.325	0.228	0.329
M	40	0.120	0.317	0.302	0.277	0.061	0.069	0.147	0.220
F	37	0.170	0.140	0.193	0.128	0.177	0.138	0.223	0.191
M	20	0.175	0.482	0.261	0.527	0.118	0.108	0.162	0.067
M	35	0.223	0.127	0.416	0.384	0.184	0.159	0.225	0.204
F	23	0.192	0.176	0.111	0.180	0.151	0.151	0.156	0.208
M	42	0.128	0.151	0.116	0.144	0.166	0.164	0.178	0.180
M	39	0.144	0.327	0.275	0.249	0.217	0.415	0.344	0.214
F	22	0.374	0.311	0.244	0.178	0.167	0.201	0.175	0.148
M	20	0.245	0.267	0.258	0.212	0.225	0.202	0.232	0.229
M	23	0.197	0.326	0.554	0.299	0.263	0.464	0.435	0.466
F	19	0.203	0.200	0.278	0.232	0.161	0.296	0.233	0.225
M	38	0.313	0.185	0.136	0.179	0.388	0.247	0.283	0.447
F	24	0.355	0.516	0.171	0.350	0.224	0.331	0.624	0.299
F	42	0.402	0.409	0.317	0.380	0.245	0.300	0.288	0.220
M	26	0.217	0.577	0.680	0.331	0.267	0.229	0.170	0.142
M	21	0.460	0.571	0.695	0.409	0.412	0.610	0.502	0.502
F	43	0.806	0.331	0.483	0.386	0.157	0.125	0.703	0.210
F	22	0.330	0.265	0.452	0.367	0.233	0.155	0.182	0.171
M	39	0.255	0.205	0.154	0.227	0.194	0.223	0.191	0.293
F	34	0.127	0.429	0.247	0.266	0.240	0.181	0.214	0.159

Table 2: Anterior rhinomanometry records of total nasal resistance before and after Drug-Test administration.

Gender	Age	Anterior Rhinomanometry - Total nasal resistance (Pa/mL/min)							
		Before Drug-Reference				**After Drug-Reference**			
		Basal	15 min	30min	60min	Basal	15 min	30min	60min
F	37	0.105	0.150	0.109	0.113	0.163	0.147	0.122	0.096
M	26	0.294	0.237	0.178	0.155	0.131	0.145	0.274	0.138
M	33	0.458	0.418	0.139	0.114	0.188	0.241	0.166	0.191
F	24	0.239	0,287	0.256	0.234	0.362	0.272	0.270	0.292
F	23	0.143	0.404	0.221	0.171	0.143	0.404	0.221	0.171
M	40	0.506	0.218	0.067	0.059	0.119	0.070	0.064	0.062
F	37	0.247	0.174	0.264	0.243	0.154	0.185	0.237	0.158
M	20	0.314	0.413	0.214	0.232	0.219	0.369	0.170	0.237
M	35	0.125	0.128	0.240	0.186	0.307	0.183	0.132	0.271
F	23	0.224	0.223	0.205	0.190	0.208	0.189	0.216	0.178
M	42	0.159	0.195	0.205	0.201	0.166	0.166	0.168	0.171
M	39	0.506	0.226	0.265	0.252	0.215	0.222	0.257	0.238
F	22	0.242	0.265	0.292	0.245	0.165	0.171	0.159	0.168
M	20	0.192	0.307	0.272	0.278	0.264	0.188	0.235	0.173
M	23	0.214	0.113	0.276	0.207	0.162	0.259	0.398	0.472
F	19	0.135	0.115	0.239	0.179	0.169	0.169	0.155	0.149
M	38	0.360	0.130	0.651	0.261	0.178	0.131	0.265	0.159
F	24	0.411	0.203	0.265	0.659	0.170	0.339	0.989	0.507
F	42	0.456	0.398	0.543	0.450	0.493	0.240	0.151	0.177
M	26	0.198	0.364	0.412	0.265	0.222	0.315	0.267	0.217
M	21	0.916	0.643	0.353	0.789	0.391	0.312	0.628	0.345
F	43	0.236	0.235	0.354	0.207	0.258	0.181	0.210	0.125
F	22	0.150	0.157	0.142	0.216	0.185	0.242	0.216	0.220
M	39	0.352	0.191	0.220	0.312	0.139	0.193	0.154	0.171
F	34	0.199	0.269	0.245	0.326	0.215	0.226	0.309	0.233

Table 3: Anterior rhinomanometry records of total nasal resistance before and after Drug-Reference administration.

Bioequivalence analysis

The bioequivalence analysis is represented by area under the curve (AUC_{0-t}) that shows the total nasal resistance variation after histamine nasal challenger at the times: before and after both treatments. The data show reduction in total nasal resistance for both treatments (test and reference drugs) in comparison with pre-treatment. Moreover, the analysis of histamine nasal challenge at 0, 15, 30 and 60 minutes suggest that both, Test and Reference drugs, present a similar protective effect

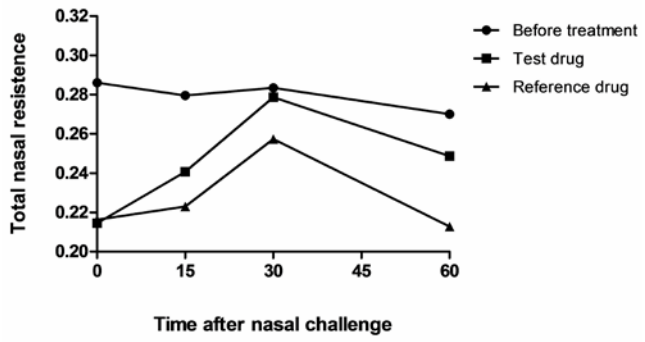

Figure 1: Total resistance after nasal challenge with histamine, comparing the measurements before and after treatment of both Test-Drug and Reference-Drug.

P value: 0.3095	Test Drug	Reference Drug
Coefficient of variation	9.57%	7.86%
Geometric mean	0.2451	0.2259
Lower 90% of geometric mean	0.2236	0.2102
Upper 90% of geometric mean	0.2686	0.2428
Geometric mean ratio $AUC_{(0-t)}$	**1.08**	

Table 4: The results of statistical evaluation –Analysis of variance ANOVA.

(Figure 1).The ANOVA of total nasal resistance for both treatments did not show a significant difference (p=0.3095) (Table 4).The geometric mean ratio (test vs reference) of AUC_{0-t} is 1.08, 90% CI 0.8 to 1.25.

Generic drug products are used for more than 50 percent of all prescriptions, and as they cost a fraction of the price of trade name drugs (drug-reference), the economic impact of Food and Drug Administration's (FDA, USA) generic drug program is intense. FDA's Office of Generic Drugs continues to make record numbers of generic products available. In 2005 alone, FDA approved 452 generic drug applications, the second highest total on record.

Nasal challenge test is an useful tools in rhinology for investigations of inflammatory mechanisms as well as for therapeutic approach of rhinitis [19]. The best nasal challenge must be able to reproduce the symptoms of rhinitis, to detect the nasal chamber modification during the test, allowing for objectives and reproductive measurements with enough sensitivity and specificity [32,33].

A nasal challenge with histamine was used in experimental models and clinical trials. Histamine is a biogenic amine synthesized and stored mainly in mast cells and basophiles. Nasal effects of histamine are mediated via interaction with H1 type receptors and H2 receptors, including: sneezing, itching of the nose, and increasing of nasal airway resistance. Histamine is the most potent mediator in both specific and nonspecific vasomotor responses. It acts both directly at the mucosal level and through nervous reflexes on vessels and glands. Histamine stimulates H1 receptors directly on the sensory nerves. Clinically, histamine causes edema and consequently nasal congestion and is considered as a good marker of nasal vasomotor response and neuroreflex. The evidence for activation of the nasal sensory afferents is intensive itching of the nose after histamine administration [33-35]. The advantage of histamine challenge that it do not evoke a significant modification of glandular secretion (advantage in rhinomanometry evaluation). Thus, the objective measures in nasal mucosa are helpful tools in rhinology to establish the relationship between stimulus and clinical symptoms [16,19,23,24,33]. Among the methods used to measure the nasal changes during nasal provocation, anterior rhinomanometry is routinely applied [33] allowing a direct evaluation of nasal patency that reflect the intensity of nasal obstruction [15,16].

In the present work the choice of anterior rhinomanometry is due to the fact that it is a quick, easy procedure and not invasive allowing an objective evaluation of nasal chamber [18,19,33,36]. The procedure is standardized, safe and reproducible; considered a gold standard for resistance to nasal flow and transnasal pressure [25,36-38].

The sneezing is a clinical occurrence after the nasal histamine challenge that can difficult an instantaneous anterior rhinomanometry measurement. Thus, we chose to evaluate the nasal patency at15, 30 and 60 minutes, defining the intermediate phase of histamine effect in nasal chamber, in opposite to immediate effect considering the time 1, 3 and 5 minutes after histamine challenge. In our previous studies (data not published), we verified the increase in nasal resistance at one hour after nasal stimulation, constituting a later effect of histamine in the nasal chamber. Working with an experimental model, Sakaguchi et al. [39] showed that nasal histamine induces a nasal obstruction at 4 hours after the nasal challenge.

In the typical bioequivalence studies, two pharmacokinetic parameters are commonly used in statistical analysis: Area Under the Curve (AUC) and maximum plasma drug concentration (C_{max}). Both parameters are obtained from the 'concentration x time' curve and reflect drug behaviour: AUC, the extent of absorption and, C_{max}, the rate of absorption. Instead of using the whole concentration profile, reducing data dimensionality is a common practice, which simplifies statistical analysis. When multivariate data is transformed into univariate data some information is left out. On the other hand, this procedure renders a special interpretation of the data, i.e., the extent of absorption.

Probabilistic distribution of both parameters generally is close to log-normal, therefore, log-transforming the data before analysis is recommended. As a matter of fact, residual analyses in general indicate the transformation.

The aim of the present study was to propose a new model to evaluate bioequivalence between nasal formulations sprays. Thus, a parameter reflecting pharmacodynamic of the drug was chosen: AUC_{0-t} from the 'resistance x time' curve similar to the pharmacokinetic parameter AUC_{0-t} from the 'concentration x time' curve in classical bioavailability studies. Residual analysis after fitting the same model used for pharmacokinetic parameters in bioequivalence studies suggested log-transformed data.

In this study, the geometric mean ratio (test vs reference) of AUC_{0-t} was1.08 (90% CI, 0.80-1.25), which fell within the bioequivalence range. The European Medicines Agency (EMEA) criterion for the pharmacokinetics' parameter defines that the 90%CI limits must be between 0.80-1.25 [40]. However, in spite of many bioequivalence studies for nasal spray drugs, the confidence intervals for therapeutic equivalence remain to be established.

The present study shows the feasibility of the nasal histamine challenge and rhinomanometry evaluation in bioequivalence studies in healthy subjects (and applicable in atopic patients) for nasal sprays, demonstrating the bioequivalence between two formulations of beclomethasone dipropionate. However, the standardization of the histamine dose necessary to promote the increase in nasal resistance in healthy volunteers is crucial. We understand that beclomethasone no longer represents the first-line corticosteroid for topical nasal administration. However, regardless of the class or type of nasal topical steroid, our purpose is to open new perspectives in bioequivalence assessment for nasal sprays as an option for the expensive clinical studies in patients with allergic rhinitis.

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