ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Original Article

Bioequivalence of 2 formulations of montelukast (5 mg chewable tablet) in the fasting state: an open-label randomized, single-dose, 2-period, crossover study

Estudo de bioequivalência entre duas formulações de comprimidos mastigáveis de Montelucaste de 5 mg em condições de jejum: um estudo randomizado, de dose única, aberto, dois períodos, cruzado

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ABSTRACT

Introduction: Montelukast, a selective and active leukotriene receptor antagonist, is one of the most common agents in asthma treatment for both adults and children. **Objective:** To assess whether the pharmacokinetic profiles of two formulations of montelukast were similar after a single 5 mg dose (chewable tablets), administered orally under fasting conditions, sampling blood before and 36 hours after administration. **Methods:** This was a randomized, 2-sequence, 2-period, crossover study of 2 chewable tablet formulations of the drug: Singulair®, provided by Merck Sharp and Dohme Farmacêutica Ltda. (reference), and generic montelukast, manufactured by Eurofarma Laboratórios S.A. (test). Plasma samples obtained from 35 participants were analyzed for montelukast through high-performance liquid chromatography coupled to tandem mass spectrometry, with montelukast-d6 as the internal standard. Peak montelukast concentrations were 299.313 (SD, 11.039) ng/mL for the reference formulation and 279.803 (SD, 10.085) ng/mL for test formulation. **Results:** Statistical analysis showed no significant differences between $AUC_{0\text{-}36h}$, $AUC_{0\text{-}inf}$, or C_{max} between formulations, with the following test/reference ratios: 102.458 for $AUC_{0\text{-}36h}$, 102.522 for $AUC_{0\text{-}inf}$, and 93.490 for C_{max}. No serious adverse events were reported during the trial. Our results demonstrated the bioequivalence of Singulair® and Eurofarma's generic montelukast.

RESUMO

Introdução: O montelucaste, um antagonista seletivo e ativo dos receptores de leucotrienos, é um dos agentes mais comumente usados na prática clínica no tratamento da asma, tanto em adultos quanto em crianças. **Objetivo:** Avaliar se os perfis farmacocinéticos de duas formulações de montelucaste eram semelhantes após uma dose única de comprimidos mastigáveis de 5 mg, administrados por via oral em jejum, coletando amostras de sangue desde antes da administração até 36 horas depois disso. **Métodos:** Estudo comparativo randomizado, 2 sequências e 2 períodos, cruzado, de dois medicamentos em comprimidos mastigáveis: Singulair®, fornecido pela Merck Sharp e Dohme Farmacêutica Ltda. (referência) e Montelucaste 5 mg, fabricado pela Eurofarma Laboratórios S.A. (teste). Amostras de plasma obtidas de 35 indivíduos elegíveis foram analisadas para montelucaste por cromatografia líquida de alta eficiência acoplada a espectrometria de massa em tandem, tendo Montelucaste-d6 como padrão interno. As concentrações máximas de montelucaste foram 299,313±11,039 ng/mL para referência e 279,803±10,085 ng/mL para formulação de teste. **Resultados:** A análise estatística não mostrou diferenças significativas para $AUC_{0\text{-}36h}$, $AUC_{0\text{-}inf}$ e nem para C_{max} entre as formulações, apresentando as razões Teste/Referência: 102,458 para $AUC_{0\text{-}36h}$, 102,522 para $AUC_{0\text{-}inf}$ e 93,490 para C_{max}. Nenhum evento adverso grave foi relatado

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Conclusions: Our results revealed that the new generic tablets are clinically safe and can be used interchangeably with the brand name product. Eurofarma's montelukast offers a safe, effective, and cheaper treatment option for people with asthma or allergic rhinitis.

Keywords: Montelukast, pharmacokinetics, asthma, therapeutic equivalence.

durante o estudo. De acordo com a regulamentação brasileira, o atual estudo farmacocinético demonstrou bioequivalência entre os agentes individuais e forneceu evidências de bioequivalência entre Singulair® e Montelukast fabricado pela Eurofarma. **Resultado:** Os resultados mostraram que os novos comprimidos genéricos são clinicamente seguros e podem ser trocados pela marca original. O montelucaste da Eurofarma oferece uma opção de tratamento mais barata, segura e eficaz para indivíduos com asma ou rinite alérgica.

Descritores: Montelucaste, farmacocinética, asma, equivalência terapêutica.

Introduction

Leukotrienes are synthesized *in vivo* from arachidonic acid through the action of the enzyme lipoxygenase or through transcellular metabolism by cells that do not express lipoxygenase. Leukotrienes are produced and secreted by numerous cells, such as mast cells, and their pro-inflammatory effects on the respiratory system induce inflammatory cell chemotaxis, airway hyperresponsiveness, and bronchoconstriction.[1] These phenomena are less evident in the lung parenchyma and are mediated by GPCR -like receptors, stimulated preferentially by leukotrienes D4 and D5.[2]

Montelukast is an orally administered medicine available as chewable tablet, oral granules, or in film-coated formulations, and has been used to treat asthma, allergic rhinitis, and exercise-induced bronchoconstriction and can be administered in adults and children aged ≥ 6 months.[3-5]

Montelukast is a photolabile molecule produced as a powder with the following International Union of Pure and Applied Chemistry name: (R,E)-2-(1-((1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(2-(2-hydroxypropan-2-yl)phenyl)propylthio)methyl) cyclopropyl) acetic acid. It has been increasingly used as a treatment for seasonal and perennial allergic rhinitis.[6] Leukotriene release causes constriction of the respiratory airways. Montelukast, which blocks the action of leukotrienes, can be used in chronic asthma prophylaxis and treatment. Thus, its pharmacological effect is due to its highly selective antagonism of D4 and E4 leukotriene receptors.[7] Bioavailability studies have shown that food in the gastrointestinal tract does not affect bioavailability, eg, for immediate release tablets taken with a standard meal.[8-10]

The aim of this study was to compare the bioavailability and characterize the pharmacokinetic profile of generic montelukast relative to Singulair®. The generic product was developed to be as effective and safe as the original brand.

Methods

Study design and participant eligibility

This was a randomized 2 x 2 crossover trial of 2 medications: Singulair® and generic montelukast. Commercially available montelukast (Singulair 5 mg, Merck Sharp & Dohme, São Paulo, SP, Brazil) and montelukast (Eurofarma S.A., São Paulo, SP, Brazil), were tested. The medications were administered orally to fasting healthy male and female volunteers, who received both treatments alternately. Each medication was tested sequentially in 2 periods: a single dose was administered with fresh water at approximately 7:00 am; the volunteers remained in the hospital the preceding night, having fasted for at least 8 h. There was a washout period of 7 days between the medications.

The clinical and statistical phases of the study were conducted at the Centro Avançado de Estudos e Pesquisas Ltda (Campinas, SP, Brazil), while the analytical phase was conducted at MAGABI Pesquisas Clínicas e Farmacêuticas Ltda. (São Paulo, SP, Brazil). This study was conducted in accordance with guidelines for clinical, laboratory, and statistical procedures, Brazilian regulations for research in human subjects, and Brazilian health authority (ANVISA) bioequivalence guidelines. All participants provided written informed consent prior to enrollment. The study was approved by the Investiga

Instituto de Pesquisas Ethics Committee (CAAE 45147721.3.0000.5599). Only adults were enrolled in the trial, primarily due to the expected pharmacokinetic similarity between adults and children,[11] as well as to ethical concerns about unnecessarily exposing children and adolescents to treatment and blood sampling.[12]

The eligibility criteria were: men and women aged 18-55 years; non-smokers (or maximum 5 cigarettes per day); body mass index between 18.5 and 29.9 kg/m^2; negative serum tests for HIV-1, HIV-2, hepatitis B, and hepatitis C; normal hemogram values; normal leukocyte and platelet counts; normal urinalysis; normal serum levels of creatinine, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, fasting glucose, and cholesterol; negative for SARS-CoV-2; in addition to normal beta-HCG labels for women. The main exclusion criteria were: a history of allergy to montelukast or related drugs; any evidence of organ dysfunction; a history of gastrointestinal, hepatic, renal, cardiovascular, pulmonary, neurological, or hematological disease, diabetes, glaucoma, psychiatric disorders, psychotropic drug or alcohol abuse; the use of any drug substances in the 14 days prior to the study; participation in another clinical trial in last 6 months; recent blood donation (< 3 months), or lack of adequate venous access.

Blood collection and analysis

Blood samples (7.5 mL) were drawn into lithium heparin vacuum tubes prior to drug administration (time 0) and at 00:20 / 00:40 / 01:00 / 01:20 / 01:40 / 02:00 / 02:20 / 02:40 / 03:00 / 03:20 / 03:40 / 04:00 / 04:30 / 05:00 / 06:00 / 08:00 / 10:00 / 12:00 / 24:00 and 36:00 (h:min) after drug administration. The samples were centrifuged at 3500 rpm for 10 min at 4 °C, handled under yellow light, and the plasma was separated and stored at -70 °C in amber flasks until transfer to the analytical facility.

Sample analysis

The plasma samples were analyzed for montelukast levels using high performance liquid chromatography coupled to an API5000 mass spectrometer (Sciex, Concord, ON, Canada). Electrospray ionization detection (positive ion mode) was used to ionize the analytes and montelukast-D6, the internal standard. The samples were extracted (50 µL added to 50 µL of a 200 ng/mL montelukast-D6 solution) through protein precipitation with methanol. After precipitation, the samples were vortex-mixed for 3 min and centrifuged at 20,800 RCF for 5 min. Five µL of the supernatant was injected into an Agilent 1200 Chromatography System (Agilent, Santa Clara, CA, USA). The samples were processed in a low light environment to avoid isomeric transformation of the drug. The chromatographic conditions for the analyte were an Agilent, Zorbax Eclipse XDB, C18, 1.8 µm (20 x 4.6 mm) column at 0.75 mL/min and, as the mobile phase isocratically pumped, a mixture of methanol/water + 23 mM formic acid (85/15; v/v). The total run time was 3.0 min. The retention times for each analyte were 1.55 min for montelukast and montelukast-D6. The mass spectrometry parameters were: dwell time 200 ms, collision energy 43 V, and collision exit energy 28.6 V for both the analyte and the internal standard. The source temperature was 600 °C; the flow rates for curtain gas, ion source gas 1, and ion source gas 2 were 10, 40, and 60 psi, respectively; and the ion spray voltage was 5500 V. Data acquisition and analysis were performed in Analyst 1.4.2. Montelukast was quantified using the multiple reaction monitoring m/z ratio (montelukast: 586.1 > 278.1; montelukast-D6: 592.4 > 278.1).

Pharmacokinetic analysis

Values for peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were taken directly from the observed concentration-time profiles. The area under the curve (AUC) for plasma concentration vs time from 0 to the last measurable concentration (AUC_{0-t}) was calculated with the linear trapezoidal rule. The AUC for 0 to infinity (AUC_{0-inf}) was calculated as AUC_{0-t} + Ct/Kel, where Ct is the last measurable concentration and Kel is the elimination rate constant.

Statistical analysis

For the relative bioavailability analysis, AUC_{0-t} and C_{max} were considered the primary variables. The analysis was performed in R. Component bioequivalence was assessed with analysis of variance and 90% confidence intervals for the test/reference ratio, using log-transformed data for C_{max} and AUC_{0-t}. The formulations were considered bioequivalent if the confidence interval for the ratio of the means was within the interval (0.80-1.25) or if the 90% confidence interval for the difference in means on the natural log scale was within the interval.

Results

Population

A total of 36 eligible healthy volunteers (18 male and 18 female) were enrolled in the study, but 1 woman dropped out (premature termination). Their ages ranged from 18 to 51 years, and their body mass index ranged from 19.63-29.81 kg/m^2.

Pharmacokinetic results

The bioanalytical method used to quantify montelukast in human plasma proved to be fast, accurate, and sensitive. The lower limit of quantification (LLOQ) for montelukast was 1.00 ng/mL. The concentration range for the calibration curves was linear from 1.00 to 1000.00 ng/mL.

The bioanalytical method was validated for precision, accuracy, recovery, specificity/selectivity, and biological matrix stability for each analyte. All results were in accordance with international bioanalytical method validation guidelines.

The mean (SD) plasma pharmacokinetic measures obtained after oral administration of a single dose of both the reference and test formulations are shown in Table 1. The mean plasma concentrations of montelukast are shown in Figure 1.

Table 2 shows the test/reference ratios and 90% confidence intervals for bioequivalence regarding AUC_{0-t} and C_{max} (log-transformed data). The statistical analysis showed no significant differences in AUC_{0-25h} or C_{max} between the formulations, demonstrating bioequivalence according to the selected criteria (90% CIs within 0.80 and 1.25). The mean AUC_{0-36h}/AUC_{0-inf} ratios were 0.990 and 0.989 for the reference and test formulations, respectively, indicating that the sampling time was adequate for both drugs.

Discussion

Our results show that both montelukast formulations had similar pharmacokinetic behavior after a single dose. The statistical analysis confirmed 90% confidence intervals for C_{max} and AUC_{0-t}, which are within the predefined limits of 80.00-125.00%, according to ANVISA and international guidelines. Wong et al. developed a chromatographic method with a faster run time but a higher LLOQ (3.0 ng/mL),[9] while Zaid et al. found a higher LLOQ (6.1 ng/mL).[10] Our LC-MS/MS bioanalysis method for drug quantification had high sensitivity and specificity, as well as high sample throughput required for pharmacokinetic, including bioequivalence, studies.

Table 1
Pharmacokinetic measurements for the reference and test formulations of montelukast after oral administration in the fasting state

Pharmacokinetic parameters	Montelukast, n=35	
	Reference Mean (±SE)	Test Mean (±SE)
C_{max} (ng/mL)	299.313±11.039	279.803±10.085
AUC_{0-36h} (ng.h.mL-1)	1954.590±84.829	1980.755±70.081
AUC_{0-inf} (ng.h.mL-1)	1974.813±86.038	2002.386±71.201
T_{max} (h)	2.490±0.145	2.914±0.174
$t_{½}$ (h)	5.367±0.073	5.493±0.089

SE= standard error; AUC_{0-36h}= area under the curve for plasma concentration vs time from 0 to the last measurable concentration (36 h); AUC_{0-inf} = AUC from 0 to infinity; C_{max}= peak plasma concentration; SD= standard deviation; T_{max}= the time to reach C_{max}; $t_{½}$= elimination half-life.

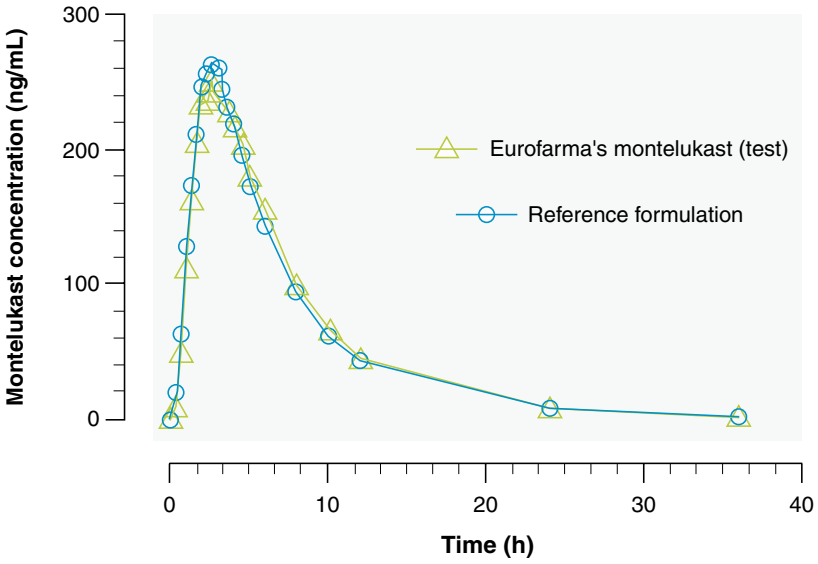

Figure 1
Mean plasma concentrations obtained in 35 participants after oral administration of Singulair (reference) and Eurofarma's montelukast (test)

Demonstrating that these montelukast formulations are bioequivalent may facilitate access to the drug. Due to the lower investment costs associated with generic or branded generic products, the introduction of Eurofarma's montelukast could have cost-effectiveness and budget implications.[13]

ANVISA and other regulatory agencies define a brand drug as a drug marketed under a proprietary, trademark-protected name, while a generic drug is similar to the brand name drug regarding the chemical formulation of the active ingredient, dosage, strength, route of administration, quality, safety, and efficacy according to the pharmacokinetic profile, and intended use. However, generic versions of a drug can vary in quality and visual appearance, including shape, labeling, and packaging. If all of the above criteria are met, then the drugs are considered therapeutically equivalent and can be safely used interchangeably.

Table 2
Pharmacokinetic measures obtained after oral administration (5 mg) of the reference and test formulations of montelukast

Parameter	N	Ratio (%)	90% CI (%)	CV (intra) (%)	Power (%)	p-value [a]
C_{max} (ng/mL)	35	93.490	(89.260 – 97.919)	11.476	100	0.779
$AUC_{0\text{-}36h}$ (ng.h.mL-1)	35	102.458	(98.431 – 106.649)	9.931	100	0.530
$AUC_{0\text{-}inf}$ (ng.h.mL-1)	35	102.522	(98.517 – 106.689)	9.869	100	0.526

[a] Partial sequence effect; $AUC_{0\text{-}36h}$= area under the curve for plasma concentration vs time from 0 to the last measurable concentration (36 h); $AUC_{0\text{-}inf}$= AUC from 0 to infinity; C_{max}= peak plasma concentration; CV = coefficient of variation.

All validation parameters were carried out according to International Conference on Harmonization guidelines and were within the accepted parameters, as shown in Table 1. Regarding the efficacy of the generic drug, statistical comparison of the main pharmacokinetic parameters (AUC_{0-36}, AUC_{0-inf}, C_{max} and T_{max}) showed no significant differences between test and reference tablets in any of the assessed parameters.

Several clinical studies have shown the efficacy of Singulair immediate release coated tablets for asthma treatment in both adults and children, as well as for symptom relief in allergic rhinitis. Since this study demonstrated the bioequivalence of the 2 formulations, Eurofarma's montelukast should have the same efficacy and safety profile.

Conclusions

Since this study has demonstrated the bioequivalence of 5 mg chewable tablets of generic montelukast and Singulair, we conclude that the two formulations can be used interchangeably. Statistical analysis of the AUC_{0-36}, AUC_{0-inf} and C_{max} results with analysis of variance showed that the test (Eurofarma) and reference (Singulair) products are bioequivalent, given that they release equivalent quantities of the active ingredient into systemic circulation at equivalent rates for both AUC_{0-36} and C_{max} ratios in the 80-125% interval.

These results show that the new generic tablets are clinically safe and can be used interchangeably with the original brand. Eurofarma's montelukast offers a safe, effective, and cheaper treatment option for people with asthma or allergic rhinitis.

Conflict of interest

Guilherme Araújo Pinto and Klaus Nunes Ficher work at Eurofarma Laboratórios S.A. All other authors declare that they have no conflicts of interest.

References

1. Foster HR, Fuerst E, Branchett W, Lee TH, Cousins DJ, Woszczek G. Leukotriene E4 is a full functional agonist for human cysteinyl leukotriene type 1 receptor-dependent gene expression. Sci Rep. 2016 Feb 2;6:20461.
2. O'Donnell SR. Leukotrienes - biosynthesis and mechanisms of action. Aust Prescr. 1999;22:55-7.
3. Sanchez G, Buitrago D. Effect of Montelukast 10 mg in Elderly Patients with Mild and Moderate Asthma Compared with Young Adults. Results of a Cohort Study. Open Respir Med J. 2018;12:67-74.
4. de Benedictis FM, Vaccher S, de Benedictis D. Montelukast sodium for exercise-induced asthma. Drugs Today (Barc). 2008;44(11):845-55.
5. Sun W, Liu HY. Montelukast and Budesonide for Childhood Cough Variant Asthma. J Coll Physicians Surg Pak. 2019;29(4):345-8.
6. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US) NCfBI. PubChem Compound Summary for CID 5281040, Montelukast 2004. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Montelukast.
7. Krell RD, Tsai BS, Berdoulay A, Barone M, Giles RE. Heterogeneity of leukotriene receptors in guinea-pig trachea. Prostaglandins. 1983;25(2):171-8.
8. Corp MSD. The prescribing information for Singulair® 2012 [Internet]. Available at: https://www.merck.com/product/usa/pi_circulars/s/singulair/singulair_pi.pdf.
9. Wong EYL, Loh GOK, Tan YTF, Peh KK. Development of LC-MS/MS method and application to bioequivalence study of a light sensitive drug montelukast. Drug Dev Ind Pharm. 2021;47(2):197-206.
10. Zaid AN, Abualhasan MN, Watson DG, Mousa A, Ghazal N, Bustami R. Investigation of the bioequivalence of montelukast chewable tablets after a single oral administration using a validated LC-MS/MS method. Drug Des Devel Ther. 2015;9:5315-21.
11. Ramakrishnan R, Migoya E, Knorr B. A population pharmacokinetic model for montelukast disposition in adults and children. Pharm Res. 2005;22(4):532-40.
12. Illamola SM, Birnbaum AK, Sherwin CM. Generic drug products in paediatrics: Where are the data? Br J Clin Pharmacol. 2019;85(9):1871-3.
13. Dunne S, Shannon B, Dunne C, Cullen W. A review of the differences and similarities between generic drugs and their originator counterparts, including economic benefits associated with usage of generic medicines, using Ireland as a case study. BMC Pharmacol Toxicol. 2013;14:1.

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