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Short Communication

Post-marketing surveillance of generic amoxicillin using a microbiological assay and pharmacokinetic approach in rats

Livia I.S. de Mattos [a], Fausto K. Ferraris [a], Tiago S.C. Machado [a], Thais M. de Brito [a], Amanda S. Chaves [a], Heliana M. Pereira [b], Douglas P. Pinto [b], Diego M.D. da Silva [b], Fabio C. Amendoeira [a,*,1]

[a] *Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz, (INCQS/Fiocruz), Av. Brasil, 4365—Manguinhos, Rio de Janeiro, RJ 21040-900, Brazil*

[b] *Laboratório de Farmacocinética, Fundação Oswaldo Cruz (Fiocruz), Manguinhos, Rio de Janeiro, RJ, Brazil*

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ABSTRACT

Generic medicines were developed to increase population access to health treatment, to reduce costs and to allow drugs with the same outcomes to be purchased at lower prices. They are therapeutically equivalent to their brand-name counterparts and are interchangeable with them. However, the acceptance of generic medicines by physicians and general consumers is often affected by distrust related to quality and efficacy. In this study three different brands of generic amoxicillin were tested. The results showed that two of them were indistinguishable from the innovator in terms of microbiological potency; however, generic B was unable to reach the Brazilian Pharmacopoeia specifications for potency limits. In contrast, generic B was bioequivalent to the innovator amoxicillin in pharmacokinetic assessment and, surprisingly, generic A, which was approved in the microbiological potency assay, lacked pharmacokinetic equivalence compared with the innovator. Both tests, when used singly, may not be effective at detecting quality deviations in antimicrobial medicines, which indicates that pharmacokinetic tests in rats in association with microbiological potency assays are a valuable tool for post-marketing surveillance of generic antibiotics.

1. Introduction

Generic drugs are medicines that are therapeutically equivalent to a brand-name counterpart and can be interchanged with it. The World Health Organization (WHO) defines these products as therapeutically equivalent if their efficacy and safety are essentially the same at the same molar dose. Equivalence has to be determined from appropriate bioequivalence, pharmacodynamic, clinical or in vitro studies [1]. The main goal of developing generic medicines is to promote the accessibility of various populations to lower-priced medicines and to increase adherence to health treatment.

Studies have indicated that government efforts to provide lower-cost treatments have been insufficient. The main barrier in low- and middle-income countries is an overall lack of knowledge about generics and the perception of many patients and physicians that generic medicines are of inferior quality [2]. In addition, a study evaluated consumer perceptions of generic prescriptions and showed that most of the population believes that this type of drug is riskier than the innovator. This line of thought is based on the knowledge that the consequences of an unsuccessful treatment are more severe when the medical condition is more serious [3].

Infectious diseases are still among the most common diagnoses in primary care, and antibiotics such as amoxicillin are used globally in the public health sector to treat bacterial infections [4–6]. Antibiotics with low quality can lead to weak treatment outcomes, thereby increasing the recrudescence potential and promoting the development of antibiotic-resistant bacteria [7]. A recent survey conducted by the Brazilian Health Surveillance Agency showed that three different brands of marketed amoxicillin presenting quality deviations have been commercially suspended [8].

Quality assessment of antibiotics in developing countries is often a two-stage process. The first stage involves drug screening, which is composed of four basic tests: visual inspection; tablet/capsule disintegration; colorimetric tests; and thin-layer chromatography. The second stage involves identifying all of the compounds present in a sample using a high-performance liquid chromatography (HPLC)

* Corresponding author. Laboratory of Pharmacology, Department of Pharmacology and Toxicology, Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz, Av. Brasil, 4365—Manguinhos, Rio de Janeiro, RJ 21040-900, Brazil. Fax: +552122900915.
E-mail address: fabio.amendoeira@incqs.fiocruz.br (F.C. Amendoeira).
[1] Present address: Laboratory of Pharmacology, Department of Pharmacology and Toxicology, Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz, Av. Brasil, 4365—Manguinhos, Rio de Janeiro, RJ 21040-900, Brazil.

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technique and then quantifying the concentration of active pharmaceutical ingredients. In addition, in vitro dissolution testing is used to predict the bioavailability and bioequivalence of tablets and capsules in vivo [5].

According to the WHO, suitable test methods to assess equivalence are comparative pharmacokinetic studies in humans, in which the active pharmaceutical ingredient (API) and/or its metabolites are measured as a function of time in an accessible biological fluid such as blood, plasma, serum or urine to obtain pharmacokinetic measures that are reflective of systemic exposure; comparative pharmacodynamic studies in humans; comparative clinical trials; and comparative in vitro tests [9]. However, the Brazilian Post-marketing Surveillance Programs performed by medicine quality control (QC) reference laboratories do not require pharmacokinetic studies for quality assurance of generic medicines. Without this study, it is not possible to guarantee correct drug availability in the systemic circulation, which could cause failure of proper therapy. For generic antibiotics, differences in their pharmacokinetic profiles can lead to variations in their clinical efficacy compared with brand-name medicines [10]. Pharmacokinetic bioequivalence evaluation of generic amoxicillin brands in the post-marketing setting is crucial to verifying their quality and what has been given to patients under treatment.

The aim of the present study was to determine the quality of different generic amoxicillin brands compared with innovators. Drug quality was assessed using a cylinder-plate assay (CPA) as well as a clinical study in rats developed for pharmacokinetic parameter evaluation and determining bioequivalence/bioavailability.

2. Materials and methods

2.1. Microbiological potency

The microbiological assessment was performed according to Brazilian Pharmacopoeia guidelines for antibiotics [11]. Potency was accepted as not less than 90% and not more than 110% of the estimated potency [11].

2.2. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis

2.2.1. Chemicals and reagents

All chemicals and reagents were prepared with type I water. Acetonitrile HPLC grade and 96% formic acid P.A. ACS were purchased from Tedia Company (Fairfield, OH), standard amoxicillin was from INCQS/Fiocruz (Rio de Janeiro, Brazil) and cefalexin was purchased from USP Pharmacopeia (Rockville, MD). The three generic brands of amoxicillin were purchased from drug stores in Rio de Janeiro (Brazil).

2.2.2. Instrumentation

A QTRAP 5500 LC–MS/MS system with MRM (AB SCIEX, Framingham, MA) was used, with ion transitions of 366.04 > 114.00 and 347.80 > 158.00. The HPLC system was Shimadzu Nexera XR LC-20AD (Nishinokyo Kuwabara-cho, Kyoto, Japan) with an Agilent Pursuit Shimadzu C18 column (150.0 mm × 3.0 mm × 5.0 μm) (Agilent Technologies, Santa Clara, CA). The mobile phase was 0.1% formic acid in type I water and acetonitrile (90:10, v/v). The flow rate was 0.8 mL/min.

Positive ionisation mode was used and the operation parameters were source temperature 400 °C; drying gas (N_2) 25 psi; curtain gas (N_2) 25 psi; and nebuliser gas (N_2) 40 psi. The ion spray voltage was 5500 V, whilst the entrance potential was 10 V. The declustering potential, collision energy and collision exit potential were 16, 27 and 8 V for amoxicillin and 61, 13 and 20 V for cefalexin. The detector operated at 1900 V.

2.3. Pharmacokinetic/bioequivalence study

2.3.1. Animals

Male Wistar rats (150–300 g) from the Fiocruz breeding colony (Rio de Janeiro, Brazil) were maintained with access to food and water ad libitum and were kept at 25–28 °C under a controlled 12-h light/dark cycle at an experimental animal facility. The Comissão de Ética no Uso de Animais (CEUA) previously approved all procedures for animal care.

2.3.2. Amoxicillin treatment

The rats were randomly separated into four groups of six animals each. The rats were fasted for ca. 12 h before drug administration. One group of rats was administered innovator and the other groups were orally administered three different brands of commercial 500 mg amoxicillin suspension (3 mg/kg), separately for each group of animals. All experimental groups received the four different drugs followed by blood sampling with a 1-week interval between administration of each drug. This time was necessary for complete restitution and drug washout, following a crossover design based on the Williams design [12].

2.3.3. Pharmacokinetic assay

Administration of oral amoxicillin (3 mg/kg) in rats was followed by blood sample collection (150 μL) at 0, 10, 20, 40, 50, 60, 120, 180, 300, 420 and 540 min after administration. Blood sampling was performed through a simple puncture in the rat tail tip with an automatic micropipette with a heparinised tip. Plasma samples were stored at −70 °C until analysis.

2.4. Statistical analysis

Pharmacokinetic parameters were calculated by non-compartmental analysis using Phoenix® WinNonlin® v.6.3 software (Certara, Princeton, NJ). Bioequivalence analysis was calculated after logarithmic transformation by applying the method of moments in individual bioequivalence, and calculation of the 90% confidence interval for the ratios in the standard average bioequivalence. The parallel lines model was used to calculate the potency of amoxicillin in the CPA. Each assay was validated by linear regression analysis, parallelism and linearity. The potency reported is the combined potency of three independent assays. Analysis of variance (ANOVA) testing was used to verify the validity of the assays. All analyses were carried out using CombiStats™ Software (European Directorate for the Quality of Medicines, Strasbourg, France). A P-value of <0.05 was considered statistically significant.

3. Results

3.1. Microbiological potency

Diameters of the zone of growth inhibition for different brands of generic amoxicillin and innovator amoxicillin are shown in Table 1. Potency was calculated as 90.8% for innovator, 95.3% for generic A, 84.0% for generic B and 92.2% for generic C.

3.2. Pharmacokinetic study

Mean concentration–time profiles of the innovator and the three generic amoxicillin brands are presented in Fig. 1.

The innovator showed an AUC_{0-t} value (area under the concentration–time curve from time 0 to the last measurable concentration) of 104,556.72 ± 6452.90 ng·min/mL, whereas generics A, B and C presented AUC_{0-t} values of 89,527.89 ± 8791.56, 99,689.59 ± 6717.48 and 108,025.08 ± 7580.78 ng·min/mL, respectively. The AUC_{0-t} of generic A was statistically inferior to that of the