

Comparative Biological Availability of Clopidogrel Formulation in Healthy Volunteers After a Single Dose Administration

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Abstract

The study was performed to compare the bioavailability of two clopidogrel 75 mg tablet formulation (Clopidogrel from Sandoz as test formulation and Plavix from Sanofi-Synthelabo Ltda, Brazil, as reference formulation) in 42 volunteers of both sexes. The study was conducted open with randomized two period crossover design and a one week wash out period. Plasma samples were obtained over a 48 hour interval. The carboxylic acid of Clopidogrel, major metabolite of Clopidogrel, was analyzed by LC-MS-MS, in the presence of enalapril maleate as internal standard. With plasma concentration vs. time curves, data obtained from this metabolite, the following pharmacokinetics parameters were obtained: AUC_{0-t} , AUC_{0-inf} and C_{max} . Geometric mean of Clopidogrel/Plavix 75 mg individual percent ratio was 100.33% AUC_{0-t} , 98.96% for AUC_{0-inf} and 105.83% for C_{max} . The 90% confidence intervals were 95.50–105.40%, 94.45–103.69%, 95.91–116.78%, respectively. Since the 90% confidence intervals for C_{max} , AUC_{0-t} and AUC_{0-inf} were within the 80–125% interval proposed by Food and Drug Administration, it was concluded that Clopidogrel 75 mg Tablet was bioequivalent to Plavix 75 mg tablet according to both the rate and extent of absorption.

Keywords: Therapeutic equivalency; Biological availability; Pharmacokinetics; Chromatography; Bioequivalence

Introduction

Clopidogrel hydrogen sulfate, methyl (+)-(*S*)- α -(*o*chlorophenyl)-6, 7-dihydrothieno [3, 2-*c*] pyridin-5(4H)-acetate hydrogensulfate, is a thienopyridine derivative that irreversibly blocks ADP receptor. Clopidogrel is chemically related to ticlopidine with superior side effects profile and dosing requirements. The drug which reduces platelet aggregation is extensively used for prevention of thrombosis in patients undergoing placement of a coronary stent (Majerus and Tollefsen, 2006). The drug is rapidly, but incompletely, absorbed after oral administration and extensively metabolized to an active metabolite. The parent drug and active metabolite are low in plasma. The major circulating compound however, is an inactive carboxylic derivative, which its blood concentration is used to document the pharmacokinetic profile of clopidogrel (Herbert et al., 1993).

Clinical studies have showed that Clopidogrel added to aspirin is beneficial in the treatment of patients with acute ST-elevation MI. Patients with unstable angina or non-ST-elevation MI should be treated with aspirin plus Clopidogrel for at least 9 months to reduce the risk vascular death, nonfatal MI, and nonfatal stroke (Aronow, 2007).

The objective of this study was to compare in healthy volunteers, the pharmacokinetics profiles and evaluate the bioequivalence of one test formulation of 75 mg tablet of Clopidogrel, elaborated by Sandoz do Brasil Indústria Farmacêutica Ltda, Brazil (test formulation). The test formulation was compared to one commercial formulation of 75 mg of Clopidogrel (Plavix) by Sanofi-Synthelabo Ltda, Brazil (reference formulation).

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 study commencement. The clinical part of the study was conducted at Scentryphar Clinical Research (Campinas City, São Paulo, Brazil) and the bioanalytical part at Nucleus of Bioequivalence and Clinical Research/NUBEC (São Paulo, Brazil).

Subjects

Forty two healthy volunteers of both sexes (21 males and 21 females) who were between the ages of 18 and 43 (mean \pm SEM: 28.7 \pm 7.6 years), who had heights between 150.0 cm and 190.0 cm (170.0 \pm 1.1 cm), and who weighed between 48.8 kg and 81.2 kg (66.7 \pm 8.1 kg) and within 15% of their ideal body weight were enrolled in the study. Subjects were judged eligible for

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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 explaining the nature and purpose of the study. The study protocol was approved by the M.M. Asser Serviços Médicos S/A Ltda with the ethical principles described in the Declaration of Helsinki, guidelines for International Conference on Harmonization-Good clinical practices (ICH-GCP).

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

Drug products

The test formulation employed was Clopidogrel 75 mg tablet (lot number KW04I04N) and the reference formulation was: Plavix 75 mg tablet (lot number 5063596).

Study Design

The study was performed to compare the bioavailability of two Clopidogrel 75 mg tablet formulation (Clopidogrel from Sandoz as test formulation and Plavix from Sanofi-Synthelabo Ltda, Brazil, as reference formulation) under fasting conditions. Formulation was tested for bioequivalence for the first time.

The study was conducted in an open randomized 2 period crossover balanced design with a 1 week wash out period between the doses. During each period, the volunteers were hospitalized at 8:00 pm having already had a normal evening meal, and after an overnight fast they received at 7:00 am a single 75 mg tablet Clopidogrel dose of either formulation. Water (200 mL) was given immediately after drug administration. All volunteers were then fasted 05 hours following the drug administration, after which a standard lunch was consumed and an evening meal was provided 10 hours after dosing. No other food was permitted during the "in-house" period. Liquid consumption was permitted ad libitum after lunch but xanthine-containing drinks including tea, coffee and cola were avoided. Systolic and Diastolic arterial pressure (measured on invasively with a sphygmomanometer automatic by Omron equipment), heart rate and temperature were recorded just before and hourly after drug administration.

Blood samples (06 mL) from a suitable antecubital vein were collected into EDTA containing tubes before and 0.167, 0.333, 0.500, 0.750, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 12.0, 16.0, 24.0, 36.0, 48.0, 72.0 hours after administration of each Clopidogrel 75 mg tablet.

Drug analysis

Blood samples were cooled in an bath and centrifuged at 3.000 rpm for at least 10 min at approximately 4°C. At least 3mL of plasma were dispensed into polypropylene tubes. Sample tubes were frozen at -20°C, and maintained to that temperature until analysis. All samples from a single volunteer were analyzed on the same day in order to avoid interassay variation.

Plasma concentrations of carboxylic acid of Clopidogrel, the

major metabolite of Clopidogrel, were determined by the HPLC coupled with tandem mass spectrometry (LC/MS/MS), using enalapril maleate as internal standard (IS). This apparatus consisted of a Shimadzu LC-10ADvp pump, and Micromass QuattroLC triple-quadrupole mass spectrometer. The analytes were extracted automatically from plasma using a SPE system consisted of a Prospekt-2™ on line solid phase extraction apparatus, using Oasis HLB Prospekt's cartridges. The validated method show a Lower limit of quantification of 10 ng/mL and linearity > 0, 98.

The analytical column was a Chromolith-RP18, 100 x 4, 6 mm, 5µ (Merck). The mobile phase used was a mixture of acetonitrile and water (50:50 v/v), containing 20 mM acetic acid. The chromatographic run time lasted 3.0 minutes with flow rate of 1.0 mL/min, in ambient temperature.

Pharmacokinetic analysis and statistical analysis

The first-order terminal elimination rate constant (Ke) was estimated by linear regression from the points describing the elimination phase on a log-linear plot, using the software SAS® Institute (Version 9.1.3). Elimination half-life ($T_{1/2}$) was derived from this rate constant ($T_{1/2} = \ln(2)/Ke$). The maximum observed plasma concentration (C_{max}) and the time taken to achieve this concentration (T_{max}) were obtained directly from the curves. The areas under the clopidogrel metabolite plasma concentration versus time curves from 0 to 72 hours (AUC_{0-72h}) were calculated by applying the linear trapezoidal rule. Extrapolation of these areas to infinity ($AUC_{0-\infty}$) was done by adding the value C_{72}/Ke to the calculated AUC_{0-72h} (where C_{72} =plasma concentration calculated from the log-linear regression equation obtained for the estimation of Ke 72 hours after dose).

The bioequivalence between both formulations was assessed by calculating individual C_{max} , AUC_{0-72h} , $AUC_{0-\infty}$ and C_{max}/AUC_{0-72h} ratios (test/reference) together with their mean and 90% confidence intervals (CI) after log transformation of the data. The inclusion of the 90% CI for the ratio in the 80% to 125% range was analyzed by nonparametric (SAS® Institute Version 9.1.3) and parametric (ANOVA) methods.

Results

Tolerability analysis

Clopidogrel was well tolerated at the administered dose. All the biochemical parameters did not any clinical relevant alterations. No adverse effects were either reported or observed.

Pharmacokinetic and statistical analysis

The mean (\pm SD) plasma concentration time profile of the 2 formulations, shown in Figure 1, was similar and superimposable.

Central and dispersion measures for all pharmacokinetic parameters for both formulations are shown in Table 1 and Table 2. From this, the mean values of C_{max} were found to be 4392.14 (\pm 1753.75 standard deviations [SD]) ng/mL for the reference product and 4469.52 (\pm 1190.44) ng/mL for the locally manufactured (test) product. For T_{max} (h), the mean values were found to be similar for both the reference and local product and the value was 1.0 (2.0) h. The mean values of AUC_{0-72} were found to be 14433.14 (\pm 3746.40) ng.h/mL for reference and

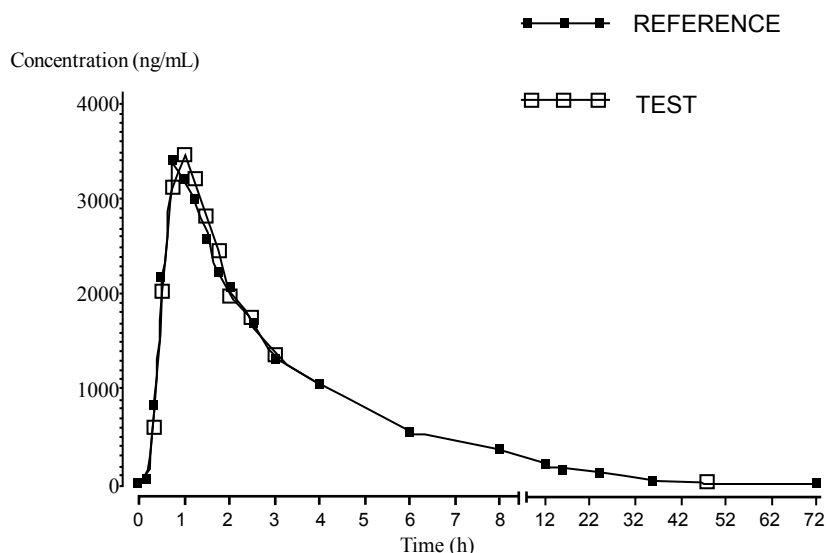


Figure 1: Clopidogrel plasma means concentration versus time profile obtained after the single oral administration of 75 mg of carvedilol formulation.

Parameter (unit)	TEST		REFERENCE	
	Mens (Median)	Standard Deviation (Amplitude)	Mens (Median)	Standard Deviation (Amplitude)
AUC ₀₋₁ (ng, h/mL)	14466.86	3714.07	14433.14	3746.40
AUC _{0-inf} (ng, h/mL)	14834.12	3734.70	14989.82	3687.03
C _{max} (ng, h/mL)	4469.52	1190.44	4392.14	1753.75
T _{max} (median/amp) (h)	1.00	2.00	1.00	2.00
Ke1 (1/h)	0.07	0.02	0.07	0.02
T _{1/2} (median/amp) (h)	9.50	13.01	9.58	18.68

Table 1: Mean pharmacokinetic parameters of clopidogrel carboxylic acid of test and reference formulation.

Parameter (unit)	TEST	REFERENCE
	Geometric Mean	Geometric Mean
AUC ₀₋₁ (ng, h/mL)	14016.94	13971.36
AUC _{0-inf} (ng, h/mL)	14395.78	14546.87
C _{max} (ng, h/mL)	4322.35	4084.09

Table 2: Geometric mean pharmacokinetic parameters of clopidogrel carboxylic acid of test and reference formulation.

Parameter	Ratio T/R (%)	Lower Limit (%)	Upper Limit (%)	Power (%)	Coefficient of variation (%)
AUC ₀₋₁	100.33	95.50	105.40	99.99	13.31
AUC _{0-inf}	98.96	94.45	103.69	99.99	12.60
C _{max}	105.83	95.91	116.78	87.49	26.91
T _{max} (dif) (h)	0.00	-0.25	0.25		

Table 3: Ratios means and the 90% geometric confidence interval of test and reference formulation.

14466.86 (± 3714.07) ng.h/mL for local product. The mean AUC_{0-∞} were found to be 14989.82 (± 3687.03) ng.h/mL and 14834.12 (± 3734.70) ng.h/mL for the reference and locally manufactured product, respectively.

Table 3 presents the ratios and the respective confidence intervals for bioequivalence analysis.

Discussion

Clopidogrel is an analogous molecule to ticlopidine and quickly binds to the platelet inhibition platelet aggregation (Bernat et al., 1993). Clopidogrel is metabolized by the liver originating its major inactive metabolite, the carboxylic acid. Due its extensive metabolization methods were developed for the quantifications in plasma of carboxylic acid.

The quantification of various drugs by chromatography with tandem mass spectrometry is if becoming each more common

time due to improvement in the sensitivity and the selectivity of this method (Takahashi et al., 2008; Bahrami et al., 2008; Ksycinska et al., 2006; Nirogi et al., 2006; Mitakos and Panderi, 2004; Shin and Yoo, 2007; Sippel et al., 2008). With the advance of the chromatography the quality in the determination of the concentrations is more precise, getting a lower LOQ and better analysis of results.

A few analytical methods have been published for determination of the inactive metabolite of clopidogrel in the biological matrix. A sensitive gas chromatography–mass spectrometric method with LOQ of 0.005 µg/mL has been published (Lagorce et al., 1998). In this technique however, a complex two steps extraction method using both liquid–liquid and solid phase extraction procedures as well as derivatization of the analyte are required. Two LC–MS methods for the determination inactive metabolite of clopidogrel have been

published (Mitakos and Panderi, 2004; Ksycinska et al., 2006). In the method described by (Mitakos and Panderi, 2004) extraction of the analyte from the serum has been achieved using single step solid phase extraction however, their method is not sensitive enough (LOQ 0.1 µg/mL) to be used in single-dose pharmacokinetic studies of the drug. In the other published LC-MS method (Ksycinska et al., 2006) two steps timeconsuming extraction procedure using both liquid-liquid and solid phase extraction techniques have been used however, the method is sensitive enough (LOQ 0.02 µg/mL) for measuring of the analyte up to at least three half lives post-dose. The inactive metabolite of the drug has been measured in Wistar rat plasma using HPLC and UV detection (Singh et al., 2005). In this method however, LOQ of 0.125 µg/mL has been reported using 50 µL injection. Furthermore, this method involves long run time of analysis (16 min) and gradient elution of the mobile phase. In HPLC-UV technique described by (Souri et al., 2006) ticlopidine has been used as internal standard. To overcome close retention times of clopidogrel carboxylic acid and ticlopidine, they used a mobile phase with low flow rate (0.9 mL/min) and high percent of aqueous phase which leads to long analytical run time (12 min) and reduction of sensitivity (LOQ 0.2 µg/mL). To improve run time and sensitivity of the analysis, the flow rate should be increased and it is preferred to select a mobile phase with higher proportion of organic solvent. Thus, comparing to their method (Souri et al., 2006), we used a mobile phase with higher flow rate and more proportion of the organic solvent. Determination of clopidogrel in human plasma by liquid chromatography/tandem mass spectrometry has been reported (Shin and Yoo, 2007). As low blood levels of the intact drug is achieved following single dose administration (Planes et al., 1999; Beom et al., 2007) and considering difference in polarity between the drug and its inactive metabolite, like other published papers (Lagorce et al., 1998; Souri et al., 2006), our method failed to detect peak of clopidogrel in the samples. However, the major advantages of the present method are reduction in run time of analysis (3.0 min) and improvement of sensitivity (LOQ 10ng/mL) which allows determination of the analyte up to three half-lives post-dose more precisely, so that the extent of absorption until the last sampling time is more closer to its extrapolated value ($AUC_{0-\infty}$).

The bioavailability of a pharmaceutical form refers to the extent and speed of absorption of the active principle in contained it. Two pharmaceutical forms are said bioequivalent when, to be administered to the same individual, in the same experimental conditions and at the same dose, showed no significant differences in relation to bioavailability. In this study two formulations of Clopidogrel had been evaluated. The mean ratio of parameters C_{max} and AUC_{0-t} and 90% confidence intervals of correspondents were calculated to determine the bioequivalence. The means AUC_{0-t} for test and reference formulation were 14466.86 ng.h/mL and 14433.14 ng.h/mL, for AUC_{0-inf} were 14834.12 ng.h/mL and 14989.82 ng.h/mL and, for C_{max} 4469.52 ng/mL and 4392.14 ng/mL, respectively. The ratios were 100.33% for AUC_{0-t} , 98.96% for AUC_{0-inf} and 105.83% for C_{max} . The 90% confidence intervals were 95.50–105.40% for AUC_{0-t} , 94.45– 03.69% for AUC_{0-inf} and 95.91–116.78% for C_{max} .

The AUC_{0-t} and $AUC_{0-\infty}$ are both recognized as an uncontaminated measurement of the extent of absorption. The

present study showed that 90% CI of mean AUC_{0-t} and $AUC_{0-\infty}$ (after log-transformation of individual ratios) were included into the bioequivalence range (80-125%), consequently, the two formulations of clopidogrel are equivalent for the extend of absorption.

The statistical comparison of C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ clearly indicated no significant difference in the two formulations of clopidogrel 75 mg tablet. 90% confidence intervals for the mean ratio (T/R) of C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were entirely within the US Food and Drug Administration acceptance range. Based on the pharmacokinetic and statistical results of this study, we can conclude that clopidogrel 75 mg tablet (Sandoz, Brazil) is bioequivalent to Plavix® 75 mg tablet (Sanofi-Synthelabo, Brazil), and that then the test product can be considered interchangeable in medical practice.

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