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Bioequivalence Study of Two Dasatinib 100 mg Formulations in Healthy Colombians

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Abstract

The presented results correspond to a pharmacokinetic study in healthy Colombian volunteers, of two Dasatinib 100 mg formulations: The Test Product is Dasatinib from Synthesis Laboratory, and the Reference Product is Sprycel® Dasatinib from Bristol-Meyers Squibb Laboratory. The design was an open, longitudinal, randomized, comparative study of two formulations in single dose of 100 mg, with a 7 days washout in between doses.

Quantification was performed using validated by Delivery Technologies Laboratories high resolution liquid chromatography, coupled to ultraviolet detector, HPLC-UV, for plasma Dasatinib identification and quantification. The main pharmacokinetic parameters were similar for both studied formulations: C_{max} 127.5 vs. 122.5 ng/mL, t_{max} 1.0 vs. 1.6 h, AUC_{0-t} 417.6 vs. 409.2 hr˚ng/mL, and AUC_{0-inf} 425.5 vs. 416.9 h˚ng/mL, Test Product vs. Reference Product.

By bringing these parameters to statistical analysis, 90% confidence intervals were found for the parameters requested by the national regulatory agency INVIMA and the internationals FDA and EMA. The 90% confidence intervals found for the relation Test/Reference, were C_{max} 84.6-116.1, AUC_{0-t} 88.2-108.9 and AUC_{0-inf} 87.9 -108.4.

According to European and FDA´s guidelines for Bioequivalence research, confidence intervals ranged between the allowed values to declare Bioequivalence and interchange ability of Test Product from Synthesis Laboratory with Reference Product.

Keywords: Bioequivalence; Dasatinib; Oncological; Pharmacokinetics

Introduction

Cancer is now a public health problem because of its impact on patients and their family's life quality, and because of the treatment high costs. Emergences of new drugs during the past 10 years, which additionally include oral administration, have eased the administration program of these therapies.

Patents expiration for many of these drugs, allow the development of generic drugs that facilitate their financing by insurance systems, but require demonstration of therapeutic equivalence.

Dasatinib orally administered selective Tyrosine Kinase Inhibitors (TKIs) that target BCR-ABL kinase and several other kinases [1,2]. It is used in adults in the treatment with Chronic Myeloid Leukemia (CML) [3,4]. In the multinational randomized DASISION trial (Dasatinib vs. Imatinib Study In-treatment-Naïve CML patients), the efficacy and safety of dasatinib were compared with those of imatinib in patients with newly diagnosed CML in the chronic phase (CML-CP) [3].

Dasatinib is a selective BCR-ABL kinase inhibitor, as well as for SRC kinase families, and other specific oncogenic kinases including c-KIT, Ephrin Kinase Receptors (EPH) and PDGF-β receptor.

It is used in adults in the treatment of CML and acute Lymphoblastic Leukemia (LLA) with positive Philadelphia chromosome (Ph^+) and blastic lymphoid crisis from CML [4].

Mechanism of action

Dasatinib, at nanomolar concentrations, inhibits the following kinases: BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRß. Besides, dasatinib is a ligand for multiple conformations of ABL kinase. Dasatinib inhibits cell lines growth overexpressing BCR-ABL in CML and acute LLA.

Dasatinib is quickly absorbed in patients after oral administration, reaching maximum concentrations between 0.5-3 h, Dasatinib's pharmacokinetics is linear in the dose range of 25 mg to 120 mg twice per day. In volunteers treated with 100 mg of dasatinib 30 min after a high-fat meal, a 14% increase in AUC was observed. The same dose given with a low-fat meal exhibited a 21% increase in AUC. These changes are not considered clinically relevant [3-5].

Dasatinib´s half-life terminal elimination is approximately 5-6 h in patients with leukemia. Dasatinib has a very large apparent distribution volume (2,505 L), suggesting that the drug is widely distributed in extravascular [3,5].

The aim of this study was to establish the pharmacokinetic profile of both Dasatinib formulations by studying and describing their bioavailability, in order to establish the speed, absorbed quantity and elimination of the active principle of interest. This with the purpose of documenting the in vivo availability of these formulations and compare their bioavailability to establish bioequivalence, following FDA and EMA's guidelines.

This article presents the results of the pharmacokinetic study in healthy Colombian volunteers, with a single dose of 100 mg, aimed to

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assess the comparative bioavailability of Test Product Dasatinib from Synthesis Laboratory, from Colombia, with Reference Product Sprycel® Dasatinib from Bristol-Meyers Squib Laboratory.

Materials and Methods

Formulations in study

Test drug: Dasatinib 100 mg coated Tablet, manufactured and distributed in Colombia by Synthesis Laboratory, Lot E-0113 D.M. 2013-03-19.

Reference drug: Sprycel® Dasatinib 100 mg coated tablet, manufactured and distributed by Bristol-Meyers Squib. Lot 3C6021B D.M. Mar/2013.

Subjects

The healthy Colombian volunteers met the inclusion criteria requested on the study protocol: volunteers from both genders, average age 28 years old, average weight 65 kg, average height 169 cm and average BMI 22.7 kg/m^2.

Before the clinical phase, the volunteers went under medical examination and laboratory tests in order to confirm their health status. In addition, women went under pregnancy test; if the result was positive, it was considered an exclusion factor.

Medical examination and laboratory tests

The requested clinical laboratory tests were: complete blood count, total and direct bilirubin, creatinine, glucose, total protein, complete urinalysis, Elisa for HIV, antibodies against hepatitis C and B, and electrocardiogram; for women, pregnancy test.

Obtaining informed consent

The Protocol and Informed Consent were authorized under code BIO 051 by the Ethics Committee in Clinical Investigation (CEIC) of Clínica de La Universidad de la Sabana, which is governed by the legal and ethical guidelines in Resolutions 008430 of 1993 [6] and 002378 of 2008 of the Ministerio de la Protección Social de Colombia [7], the World Conference on Harmonization for good clinical practices in institutions that conduct research in humans, and by the principles of World Medical Assembly published in the Declaration of Helsinki, last reviewed in 2013 [8].

Volunteers were given a talk, intended to explain in detail the study, emphasizing the drug type, dose, possible adverse reactions, amount of blood that was going to be taken for samples on each study phase, materials to obtain the samples, health team in charge of taking and supervising the samples, dietary restrictions they would face; all questions that were asked by the volunteers were solved, so they could freely decide to participate in the study. After that, each one of the volunteers signed the Informed Consent.

Study design

The design was an open, randomized, two sequences, crossed study, and a 7 days washout in between periods. Three days before the beginning of each period, volunteers had to abstain from medicines, alcohol and any food or beverage containing methylxanthines. These restrictions were maintained throughout the time that the samples were obtained. All volunteers were randomized to be assigned to the treatment sequence.

Drug administration

Before the drug administration, the volunteers kept fasting for 10 h. Then, they were given a 100 mg Dasatinib dose [9] which was ingested with 200 mL of water, 4 h later, and each volunteer was provided with standardized food. During the stay period in the clinic, three complete meals (breakfast, lunch, and dinner) and two snacks (one in the morning, one in the afternoon) were provided.

The team for obtaining the samples consisted of a doctor and a licensed nurse. With a Vacutainer®, blood samples by venipuncture from an upper limb were taken. All volunteers received either the Test or the Reference Product by randomization. 12 venous blood samples were taken at the following times: 0, -2, 0 h (immediately before drug administration), and 0.33, 0.66, 1, 1.5, 2, 2.5, 4, 6, 9, 12 years 24 h after. Samples were labelled for identification and centrifuged at 4,500 rpm during 25 min. Plasma was transferred into pre-labelled cryoviales and frozen at -20°C for further analysis.

After 7 days of washout, the same procedure was repeated, completing the second study period.

Validation of the analytical method

Validation was performed according to the bioanalytical methodology validation procedure established by Delivery Technologies (EBA13090250064).

1.0 mL of the test sample was added into an essay tube with screw cap for centrifugation, for sedimentation, that contained 3 mL of Acetonitrile and was subjected to vortex during 60 s. After that, it was centrifuged at 5000 rpm during 10 min. The supernatant was removed to a clean glass tube and evaporated to dryness at 60°C under a nitrogen gas stream. Finally, the residue was reconstituted with 1.0 mL of diluent solution and subjected to vortex for 30 s. It was filtered and 100 mL was injected into the chromatographic system.

Analytical procedure

Buffer solution preparation (monobasic potasium phosphate 25 mM, pH 6.5)

- A 4.3 g solution of dihidrogenated potasium phosphate was prepared in 1 L of water.
- 4 mL of triethylamine was added.
- pH was adjusted to 6.5 ± 0.05 with concentrated H_3PO_4.
- It was 0.45 µm membrane filtered and the diluent solution was buffer pH 6.5 and Methanol (60:40 v/v) [10-12].

Chromatographic Conditions

- Agilent liquid chromatograph, Column thermostated UV detector, ChemStation software.
- Column: C18, 150 mm × 4.6 mm, 5 µm.
- Column Temperature: 40°C.
- Detector: UV-VWD.
- Wavelength: 325 nm.
- Injection Volume: 100 µL.
- Flow: 1.0 mL/min.
- Flow rate: 15 min.
- Mobil phase: Buffer phosphate 25 mM, pH 6.5, Acetonitrile (60:40 v/v).

Pharmacokinetic analysis

The Pharmacokinetic Analysis was performed by WinNonlin 5.3 program (Pharsight Corporation, Cary USA), adjusted to a non-compartmental analysis. The maximum concentration ($C_{máx}$) and the time to reach it ($t_{máx}$) were directly obtained from the serum concentration results, as currently recommended by the FDA [13] and the EMA (European Medicines Agency) [14].

AUC_{total} was calculated by the sum of the partial AUC

a) AUC_{0-t}, between time zero and the last time with detectable concentrations, calculated by the trapezoidal rule, and ensuring the calculation of at least the 80% of the AUC with the last sample. b) $AUC_{t-\infty}$, calculated as the ratio C/K, being C the last detectable concentration and K the slope of the line, obtained by linear regression, from the points corresponding to the elimination phase of the drug, by linear regression of the natural logarithm of the concentrations [15]. The elimination rate constant (Ke), half-life ($t_{½}$), the Clearance (Cl) and the Mean Residence Time (MRT), adjusted to Bioavailability, were calculated after the non- compartmental analysis.

Statistic analysis

Variance Analysis (ANOVA) was used to determine possible effects for each variation factor, per sequence, period, or subjects. The F-test was used with a statistical significance level of 5% (α=0.05%). The statistical comparison of the transformed pharmacokinetic parameters on both formulations, was performed using the statistical program WinNonlin version 5.3.

The following bioequivalence criteria were established on the protocol: The Confidence Interval of 90% of C_{max} Test/C_{max} Reference and last test AUC/last reference AUC, relations must be in the range of 80-125% of acceptability. Plus, AUC_t must not be less than 80% of AUC_t.

Adverse events report

The adverse events were registered following the INVIMA regulations, which define them as events that occurred probably related to the study´s medication.

Results

The physical exams performed to all participant volunteers reported normal results on each one of the volunteers and this was verified by, the Medical Coordinator of the study, Dr. Edgar Augusto Villarraga, MD, MSc, who was responsible and performed physical examinations two weeks after having ended this study, which determined with lab tests that all volunteers are now in good health conditions, thereby closing their charts.

Quantification was performed using validated by Delivery Technologies Laboratories high resolution liquid chromatography, coupled to ultraviolet detector, HPLC-UV, for plasma Dasatinib identification and quantification.

Table 1 and Figure 1 show the average of the pharmacokinetic parameters obtained from all volunteers. The main pharmacokinetic parameters were similar for both studied formulations: C_{max} 127.5 vs. 122.5 ng/mL, t_{max} 1.0 vs. 1.6 h, AUC_{0-t} 417.6 vs. 409.2 hr*ng/mL, and AUC_{0-inf} 425.5 vs. 416.9 hr*ng/mL, Test Product vs. Reference Product.

Table 2 show the 90% Confidence Intervals of the logarithmically transformed pharmacokinetic parameters, analysis performed to determine Bioequivalence between Test Product from Synthesis, and Sprycel® Reference Product from Bristol-Meyers Squib, found for the relation Test/Reference, were C_{max} 84.6-116.1, AUC_{0-t} 88.2-108.9 and AUC_{0-Inf} 87.9-108.4.

Lastly, Table 3 summarizes the events that occurred, that were expected reactions in relation to the studied drug and the administered dose. No unexpected reactions happened.

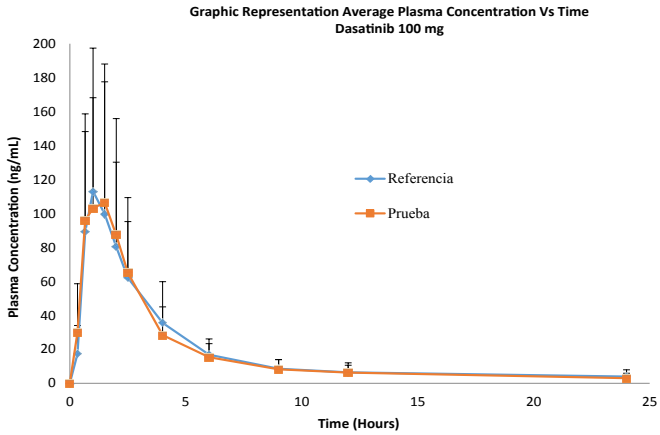

Figure 1: Bioavailability curve (Plasma Concentration vs. Time) obtained after a 100 mg Dasatinib dose in fasting conditions of test product (Synthesis) and reference product (Sprycel® from Bristol-Meyers Squib).

Dasatinib Pharmacokinetic Parameters					
Treatment	**Elimination (1/h)**	**t_{max} (h)**	**C_{max} (ng/mL)**	**AUC_{0-t} (h*ng/mL)**	**AUC_{0-Inf} (h*ng/mL)**
Test	0.170	1.0	127.5	417.6	425.5
Reference	0.189	1.6	122.5	409.2	416.9

Dasatinib pharmacokinetic parameters of test product from synthesis laboratory, Colombia, and reference product Dasatinib, Sprycel® form Bristol-Meyers Squibe laboratory, after a100 mg single dose.

Table 1: Pharmacokinetic results.

	Units	**Ratio%**	**90% Standard IC (Test/ Reference)**	
Ln (C_{max})	ng/mL	99.1	84.6	116.1
Ln (ABC_{o-t})	h*ng/mL	98.0	88.2	108.9
Ln ($ABC_{o-\infty}$)	h*ng/mL	97.6	87.9	108.4

Table 2: 90% Confidence intervals for Dasatinib formulations (Test and Reference Products) after administration to healthy volunteers.

Discussion

The presented results correspond to a pharmacokinetic study in healthy Colombian volunteers, of two Dasatinib 100 mg formulations: the Test Product is Dasatinib from Synthesis Laboratory, and the Reference Product is Sprycel® Dasatinib from Bristol-Meyers Squibb Laboratory.

Generic drugs interchange ability is demonstrated by comparative pharmacokinetic studies, conducted with Reference Products, to demonstrate no significant difference in the speed and amount of active principles delivered by the studied formulations. These studies are recognized by the various OMS's regulatory entities: EMA, FDA and INVIMA [13-16].

The analytic method was accurate and the variability in the results was due to the volunteers. Loss of samples occurred because of threw up

ADE	Reference	ADE	Test
Vomit	25%	Nausea	22%
Abdominal pain	19%	Abdominal pain	19%
Diarrhea	19%	Diarrhea	19%
Headache	14%	Vomit	8%
Nausea	14%	Headache	8%
Dizziness	3%	Dizziness	6%

Table 3: Summarized ADE presented by studied formulation.

in the volunteers; that information was taken away from the analysis, in order to prevent variables in the absorption process of the studied active principle.

The adverse reactions that were presented were expected in all cases and did not require the use of medications

Conclusion

Dasatinib formulation manufactured by Synthesis, Test Product, and formulation manufactured by Bristol-Meyers Squib (Sprycel®), Reference Product, has pharmacokinetic parameters that allow declaring Bioequivalence between both formulations.

This study demonstrated that Dasatinib formulation from SYNTHESIS laboratory, Dasatinib®, is an adequate formulation to perform delivery of the active principle in an effective and safe way.

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