Bioequivalence Study of Two Formulations Containing Bosutinib 500 mg Tablets in Healthy Colombian Volunteers

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Abstract

To analyze the bioavailability of the Test product (Bosutinib 500 mg, made by Laboratorios Lafrancol S.A., Colombia) by correlating with the Reference product (Bosulif® made by Pfizer), the pharmacokinetic study of the two formulations were done. This study will affirm the bioequivalence between the same. A crossover study was carried out with 36 healthy volunteers where a single 500 mg dose was administered during fasting condition, having 15 days washout period and 12 plasma samples were collected within 0-72 h. The trial was open-label and two period and two sequence randomized earlier. High-resolution liquid chromatography method was used for analysis and mass spectrometry detector tandem HPLC MS/MS was done to identify and quantify the plasma Bosutinib. The parameters considered during the pharmacokinetic study are as follows: The statistical analysis of these parameters determines the confidence intervals to 90% as appealed by the FDA and EMA international and national regulatory agencies (INVIMA).

As per the European and FDA guidelines for Bioequivalence research, the Confidence Interval is within the granted the Bioequivalence criteria affirmation and correlation of the Lafrancol S.A. product between the Reference product.

Keywords: Bioequivalence; Bosutinib; Cholesterol; Statin; Pharmacokinetics

Introduction

Bosutinib can be categorized under subclass of drugs, known as kinase inhibitors. The abnormal kinase Bcr-Abl is inhibited Bosutinib. The study models explained the conjugation of bosutinib and the kinase domain of Bcr-Abl. The Src kinase family, including Src, Lyn and Hck can also be inhibited by Bosutinib, and PDGF receptor and c-Kit are minimally inhibited [1].

Adult patients suffering from chronic myeloid leukemia with Philadelphia chromosome positive (Ph+CML) in Chronic Phase (CP), Accelerated Phase (AP) or Blastic Phase (FB) are advised to be treated with Bosutinib, previously treated with one or more inhibitors tyrosine kinase and for whom treatment with imatinib, nilotinib and dasatinib are not considered appropriate [2]. A physician should be experienced in the diagnosis and treatment of CML, to treat such patients (Tables 1 and 2).

This study aims at establishing the bioequivalence of the constituents having Irbesartan 500 mg tablets by relating the bioavailability, after one dosage, of the test product by Lafrancol S.A. (Colombia) and the Reference product, Bosulif®, produced by Pfizer.

Materials and Methods

Study formulations

Test Drug: Bosutinib 500 mg tablets, produced in Colombia by Lafrancol S.A. Lot 4A2995 F: F 2014/02/24

Reference Drug: Bosulif® Bosutinib 500 mg tablets, produced and dispersed by Pfizer. Lot H55746 F: Exp 10/2015

Subjects: 36 healthy individuals, 18 females and 18 males, who does not smoke, with a mean age of 33 years old, range between 22 and 51 years with an average Body Mass Index (BMI) of 22.6 kg/m² (15.4-25.8 kg/m²) and an average height of 158 cm (145-168 cm) concluded the research (Table 1).

To confirm the health status, medical assessment, medical examination and laboratory tests were done for all volunteers prior to the clinical stage. The exclusion factors that were considered were alcoholism history, pre-existing diseases like weak liver or kidney function, blood dyscrasia or proteinuria.

Medical evaluations and clinical trials: Clinical laboratory tests that were performed includes total count of blood, whole and direct bilirubin, creatinine, glycaemia, whole protein, complete urinalysis, HIV-ELISA test, antibodies against hepatitis B and C, electrocardiogram and blood pregnancy test for women.

Informed consent process: The agreement and the briefed authorization form were entitled by the La Sabana University Clinical Research Ethics Committee (CREC) which is administered by the legal and ethical guidelines of the decisions such as 008430 from 1993 and 002378 from 2008 of the Ministry of Social Protection (Colombia).

Also, by World Conference on Harmonization for Good Clinical Practice of Institutions Conducting Investigation in Human Subjects and by the World Medical Assembly principles advertised in the Declaration of Helsinki, last review in 2008 [2].

The study, signifying the medication type, dosage, possible drug adverse reactions, collection of blood samples at intervals, type of materials to be used for sample collection, examining and controlling...
stuff, restrictions in diet and all the data, as appealed by the volunteers, was elucidated to them so that they willingly decide on their cooperation in the study. Hence, each subject's signature was taken in the informed consent form.  

Study design

An open labeled, double sequenced, two periods randomized crossover model was taken during a 15 days washout period between periodic intervals. Three days prior to each periodic induction, volunteers were deprived of any medication, alcohol and any food items consisting of methylxanthines. These restraints were continued throughout the sampling process. A randomized allocation of all volunteers was done for the treatment sequence.

Drug administration

Volunteers were deprived of any medication or food 10 h prior to the drug administration. The drug was given with 200 mL of water as a single dosage of 500 mg Bosutinib [3], (i.e., 1 tablet of 500 mg) to each volunteer, and then after two h, each volunteer could have standardized food. During the stay at the hospital, the subjects were given three times a day at 08.00, 12.00 and 18.00 h, each volunteer could have standardized food. Such sample is known as “zero time point sample”.

The sampling team comprised of a doctor and one registered nurse. Blood specimen was collected by using Vacutainer®, which was centrifuged at 4000 rpm for 25 min. Previously labelled tube was transferred with Plasma and frozen at -20°C for analysis later. After 15 day washout period, deliverance was done again for the next study period [4].

Validation of analytical method

The validation was carried out according to the procedures described in “Validation of bioanalytical methodology” by QUASFAR M & F S.A. (PL-021). (Table 2)  

Accuracy: ±15% of the nominal concentration.

AUC total was determined by the sum of fragmented or partial AUC

a) AUC<sub>0-t</sub>, between zero time point and the last time point with distinguishable concentrations, determined by the trapezoidal rule and assuring the computation of 80% of the AUC with the end sample.

b) AUC<sub>0-∞</sub> computed from the C/K ratio, where C is the end detectable concentration and K is the slope given by linear regression from the points analogous to the drug elimination stage given by a linear regression of the common logarithm of concentrations [8].

Bioavailability-accustomed elimination constant (K<sub>e</sub>), half-life (t<sub>½</sub>), clearance (Cl) and mean residence time (MRT) were determined by applying the non-compartmental analysis. The outcomes of the pharmacokinetic parables are compiled in Table 3 with the Clearance, half-life, C<sub>max</sub>, AUC<sub>0-∞</sub>, T<sub>max</sub> values and the elimination rate (K<sub>e</sub>) of each formulation that has been examined.

Statistical analysis

An Analysis of Variance (ANOVA) was applied to resolve probable outcomes for each varying factor with respect to sequence, period or subject. For this, F-test with a statistical significance level of 5% (α=0.05) was applied. Statistical correlation of altered pharmacokinetic criterions of both formulations was carried out applying the statistical software WinNonlin (version 5.3). The Bioequivalence level was stated in the protocol follows as: The 90% confidence interval of Test C<sub>max</sub>/Reference C<sub>max</sub> and end Test AUC/last Reference, ratios that should be between the range 80-125% acceptability. In addition, the end AUC criterias should not be less than 80% of total AUC criteria [9].

Adverse events report: INVIMA Framework Provision No. (1067/08), states the detrimental recorded events, and defines them as severe as well as unserious and probable or non-related to the study

<table>
<thead>
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<th>Kinetic parameters compared study Bosutinib</th>
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<tr>
<td>Treatment</td>
<td>Elimination (t&lt;sub&gt;½&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Test</td>
<td>0.02</td>
</tr>
<tr>
<td>Reference</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 1: Pharmacokinetic results.

| Reference product and 12 samples of venous blood were collected per volunteer, and then after two h, each volunteer could have standardized food. During the stay at the hospital, the subjects were given three times a day at 08.00, 12.00 and 18.00 h, each volunteer could have standardized food. Such sample is known as “zero time point sample”.

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<table>
<thead>
<tr>
<th>Adverse events</th>
<th>RAM</th>
<th>Reference</th>
<th>RAM</th>
<th>Prueba</th>
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<tbody>
<tr>
<td>Threw Up</td>
<td>25%</td>
<td>Sickness</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>19%</td>
<td>Abdominal Pain</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>19%</td>
<td>Diarrhea</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>Cefalea</td>
<td>14%</td>
<td>Threw Up</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Sickness</td>
<td>14%</td>
<td>Cefalea</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Drowsiness</td>
<td>3%</td>
<td>Drowsiness</td>
<td>6%</td>
<td></td>
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Table 3: Summary submitted by formulation studied RAM.
medication. As the sample amount, do not hold enough statistical potential, the cases are reported as obtained from the examination unit without any statistical evaluation (Table 3).

Results

36 healthy Colombian volunteers comprising of 50% women and 50% men where 35 among them completed both trials and were considered in the pharmacokinetic and statistical analysis [10,11].

(Table 1) The averages of the parameters considered during the pharmacokinetic study obtained from the subjects (mean ± SD) are shown whereas in Table 2 the Confidence Intervals of 90% of the pharmacokinetic parameters logarithmically altered, and analysis carried out to resolve the bioequivalence between the test of Lafrancol S.A. and Bosulif® produced by Pfizer are shown in Table 3.

Discussion

The bioequivalence studies are accepted as proof surrogate by WHO, EMA, the FDA and INVIMA that allows us to think with a great probability regarding the two drugs having efficacy and similar safety profile, i.e., therapeutic equivalency [11]. Healthy Volunteers with single dose regimen were considered for the trials in this pharmacokinetic study. The study aimed at achieving the bioequivalence of two Bosutinib 300 mg formulations. The analysis of bioequivalence pharmacokinetic parameters was performed by considering Bosutinib plasma concentrations against time, after examination of blood samples obtained from the healthy volunteers (Figure 1).

Conclusion

The parameters considered during the pharmacokinetic study, establishes the bioequivalence between both formulations namely Bosutinib manufactured by Lafrancol S.A.S (Test Product) and Pfizer manufactured by Bosulif (Reference Product).

References

3. WMA Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects (2013) 64th WMA General Assembly, Fortaleza, Brazil.