Comparative study of relative bioavailability between two formulations containing Clozapine 100 mg in patients with schizophrenia

Estudo comparativo de biodisponibilidade relativa entre duas formulações contendo Clozapina 100 mg em pacientes portadores de esquizofrenia

Pablo Rezende de Oliveira¹, Leonor Garcia Rincon², Paula Rocha Chellini³, Juliana Baratta⁴, Arminda Lucia Siqueira⁵, Thiago Horta Soares⁶, Marco Túlio Baccarini Pires⁷

¹ Student at the Medical School of the Federal University of Minas Gerais – UFMG, Trainee in the Biotechnology Research Center – CEBIO. Belo Horizonte, MG-Brazil.
² MD, Cardiologist. PhD in Tropical Medicine. Coordinator of the clinical studies phase in bioequivalence studies at CEBIO. Belo Horizonte, MG – Brazil.
³ Pharmacist and Biochemist. PhD student in medicine and cosmetic quality control at the Federal University of Minas Gerais. Belo Horizonte, MG-Brazil.
⁴ Pharmacist. Master’s degree student at the Federal University of Minas Gerais. Belo Horizonte, MG – Brazil.
⁵ Statistician. PhD in Biostatistics. Professor of the Department of Statistics (DEST) of UFMG. Belo Horizonte, MG – Brazil.
⁷ MD. PhD in Surgery. Principal Investigator of CEBIO. Medical Director of Bibliomed – Medical Virtual Library. Belo Horizonte, MG-Brazil.

Conflict of interest - This study received funding from Lifal – Industrial Pharmaceutical Laboratory of Alagoas S/A. However, the results were not influenced.

DOI: 10.5935/2238-3182.20150012

ABSTRACT

This study aimed to evaluate the bioequivalence between two products containing Clozapine 100 mg (testing product: LifalClozapina® from Lifal – Industrial Pharmaceutical Laboratory of Alagoas S/A and the reference product: Leponex® from the Novartis Biociências S/A. Laboratories) in 40 volunteers. The study was open, randomized, cross-over type, in a state of equilibrium, with two periods (two sequences) in which volunteers were given, in each period, the testing formulation or the reference formulation. The relative bioavailability of the formulations following oral administration was evaluated based on statistical comparisons of relevant pharmacokinetic parameters obtained from blood samples in a 24 hours period. The concentration of Clozapine was measured using an appropriate and valid analytical method. The pharmacokinetic measures used were: Cmin and Cmax, and ASCt. The mean differences (± SD) between reference and testing were: 0.1615 ± 0.3404 (ng/mL); -0.0969 ± 0.4131 (ng/mL); and 0.9143 ± 3.9941 (ng*h/mL). The confidence intervals for medium bioequivalence met the limits determined by the National Agency of Sanitary Surveillance (ANVISA) of 80 to 125%, and both medications were considered bioequivalent. Thus, the testing and reference products are considered interchangeable.

Key words: Biological Availability; Therapeutic Equivalency; Clozapine; Pharmacokinetics; Schizophrenia.

RESUMO

Este estudo teve por objetivo avaliar a bioequivalência entre dois produtos contendo Clozapina 100 mg (produto teste: LifalClozapina® da Lifal – Laboratório Industrial Farmacêutico de Alagoas S/A. O produto referência: Leponex® do Laboratório Novartis Biociências S/A.) em 40 voluntários. O estudo foi aberto, randomizado, do tipo cross-over, em estado de equilíbrio, com dois períodos (duas sequências), nos quais os voluntários receberam, em cada período, a formulação teste ou a formulação referência. A biodisponibilidade relativa das formulações seguidas a administração oral foi avaliada com base em comparações estatísticas de parâmetros farmacocinéticos relevantes obtidos de dados de amostra sanguínea dos voluntários, sendo as amostras coletadas em período de 24h. A concentração de Clozapina foi medida a partir de método analítico apropriado e válido. As medidas farmacocinéticas utilizadas foram: Cmin, Cmax e ASCt. As diferenças médias (± DP) entre referência e teste foram: 0.1615 ± 0.3404 (ng/mL); -0.0969 ± 0.4131 (ng/mL); e 0.9143 ± 3.9941 (ng*h/mL). Os intervalos de confiança para biequivalência média atenderam aos limites determinados pela Agência Nacional de Vigilância Sanitária (ANVISA) de 80 a 125%, sendo os dois medicamentos...
Comparative study of relative bioavailability between two formulations containing Clozapine100 mg in patients with schizophrenia

INTRODUCTION

Clozapine is an atypical antipsychotic used in the treatment of schizophrenic patients, acting against negative and positive symptoms of the disease. The atypical agents are indicated as first-line medications for patients that are resistant to the usual treatment, have a predominance of negative symptoms, and are showing dystonia or extrapyramidal effects, or suicidal tendencies, and is considered more effective than other therapeutic options in such cases.

In Brazil, the use of Clozapine is indicated only after therapeutic failure with two typical antipsychotics, followed by failure in the use of risperidone (atypical agent with potency and price lower than that of Clozapine) due to its hematological adverse effects. The administered dose of this medicine should be individualized, always adopting the lowest for each patient, for safety and reduced incidence of side effects.1-8

The limitations to the use of Clozapine are its side effects (rare but severe) including agranulocytosis that affects less than 1% of users. In addition, it is a very expensive drug in Brazil and not accessible to the majority of the population.

The reference medicine sold in the Brazilian market is Leponex®.1-10 In order to launch a new drug (generic drug) with lower cost and competitiveness with this drug, research on the relative bioavailability (extent and speed of drug absorption) between the reference and the new drug must be conducted comparing behaviors in the human body.

This study aimed to evaluate the bioequivalence of two products containing Clozapine. The testing product was 100 mg Clozapine pills (LifalClozapina®) from Lifal-Industrial Pharmaceutical Laboratory of Alagoas S/A. The reference product was 100 mg Clozapine pills (Clozaril®) from Novartis Bioscience Laboratory. The study was performed on individuals with schizophrenia already using this drug.

Because bioequivalence studies involve the handling of pharmaceuticals and research with human beings, the regulations set by the National Agency of Sanitary Surveillance (ANVISA) and Resolution RE nº 1,170 of 2006 were followed. The study was conducted in the Biotechnology Research Center (CEBIO) and the hospitalization center from the Belo Horizonte Hospital, in Belo Horizonte, Minas Gerais.11

MATERIAL

A total of 2,400 pills of the reference drug (Leponex® from the Novartis Bioscience Laboratory S/A-Z0108 batch and expiration date on May 2010) and 2,400 pills of the testing drug (LifalClozapina® from Lifal-Industrial Pharmaceutical Laboratory of Alagoas S/A – 0809102 batch and expiration date on September 2010) were acquired.

METHOD

The study was designed to allow the evaluation of relevant pharmacokinetic parameters for statistical comparisons, aiming at the investigation of bioequivalence/bioavailability. In this study, such parameters were obtained directly from the determination of plasma concentration of the active ingredient in the drug based on the application of a non-compartmental model, suitable for the evaluation of these concentrations after oral drug administration.

According to the guide for bioequivalence studies on Clozapine pills from the Food and Drug Administration (FDA) of the United States of America on June 2005, and with the guidance from ANVISA, the bioequivalence study of Clozapine should not be performed on healthy volunteers. This requirement is justified by the occurrence of numerous severe adverse effects in healthy volunteers who received Clozapine in other studies, such as agranulocytosis, orthostatic hypotension, bradycardia, syncope, and asystole.12,13 The authors of this study received the approval of the Research Ethics Committee to perform this study with the selected population.

It is reported that cardiovascular effects can occur with more frequency and seriousness in healthy individuals who had used Clozapine when compared with patients with schizophrenia who had already used this drug.17

The study was open, randomized, cross-sectional (crossed), in equilibrium (steady-state-point where the drug elimination rate is equal to the bioavailability rate when there is a constant concentration in the blood) including patients with schizophrenia and already in use of Clozapine, who were recruited as...
Comparative study of relative bioavailability between two formulations containing Clozapine 100 mg in patients with schizophrenia

by a multidisciplinary team composed of physicians, nurses, pharmacists, and Nursing trainees. The aim of this multidisciplinary team was to verify the correct implementation of the study and proper management of volunteers ensuring their comfort and well-being.

The drug exchange occurred on the 11th day of the study; volunteers who were in use of the reference drug began taking the testing drug, and those in use of the testing drug began taking the reference drug. Volunteers were discharged and followed up by nurses in their homes, who always visited at the time of drug administration. These procedures were placed to ensure that the state of equilibrium was achieved until the day of blood sample collection for the bioequivalence research.

On the 16th day of the study, volunteers were admitted again at around 5 pm for the second period of the study. On the 17th day, pre-dose blood samples were collected at about 6:30 am and volunteers received one 100 mg Clozapine pill (or multiple of 100 mg) orally with 200 mL of water at around 7 am.

Blood samples were collected on the 7th, 8th, and 9th day of the study to determine if blood levels of Clozapine had reached the state of equilibrium. On the 9th day, volunteers were admitted to the Belo Horizonte Hospital at around 5 pm for the first period of hospitalization. On the 10th day, at around 6:30 am, pre-dose blood samples were collected, and volunteers received one 100 mg Clozapine pill (or multiple of 100 mg) orally with 200 mL of water at around 7 am. During hospitalization, volunteers were followed up

Table 1 - Drug Administration and sample collection

<table>
<thead>
<tr>
<th>Days</th>
<th>Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st to T</td>
<td>Testing/Reference Drugs</td>
</tr>
<tr>
<td>T to 9th</td>
<td>Testing/Reference Drugs (blood sample to evaluate equilibrium and hospitalization on the 9th)</td>
</tr>
<tr>
<td>10th</td>
<td>Testing/Reference Drugs (Administration of drug under hospitalization. Blood samples were collected after 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hs of drug administration)</td>
</tr>
<tr>
<td>11th</td>
<td>Exchanging drugs (resumed drug administration at home)</td>
</tr>
<tr>
<td>12th - 13th</td>
<td>Medicamento Teste/ Referência</td>
</tr>
<tr>
<td>14th - 16th</td>
<td>Testing/Reference Drugs (blood sample to evaluate equilibrium and hospitalization on the 16th)</td>
</tr>
<tr>
<td>17th</td>
<td>Testing/Reference Drugs (Drug administration during hospitalization. Blood samples were collected after 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hs of drug administration)</td>
</tr>
</tbody>
</table>

Table 2 - Dose used for each volunteer

<table>
<thead>
<tr>
<th>Volunteers</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID</td>
<td>Initials</td>
</tr>
<tr>
<td>1</td>
<td>FCM</td>
</tr>
<tr>
<td>2</td>
<td>CAT</td>
</tr>
<tr>
<td>3</td>
<td>WSD</td>
</tr>
<tr>
<td>4</td>
<td>PES</td>
</tr>
<tr>
<td>5</td>
<td>ITC</td>
</tr>
<tr>
<td>6</td>
<td>DAI</td>
</tr>
<tr>
<td>7</td>
<td>FFP</td>
</tr>
<tr>
<td>8</td>
<td>PHR</td>
</tr>
<tr>
<td>9</td>
<td>EFP</td>
</tr>
<tr>
<td>10</td>
<td>RRO</td>
</tr>
<tr>
<td>11</td>
<td>IMS</td>
</tr>
<tr>
<td>12</td>
<td>MLC</td>
</tr>
<tr>
<td>13</td>
<td>JVD</td>
</tr>
<tr>
<td>14</td>
<td>MAF</td>
</tr>
<tr>
<td>15</td>
<td>RBJ</td>
</tr>
<tr>
<td>16</td>
<td>MPR</td>
</tr>
<tr>
<td>17</td>
<td>EJR</td>
</tr>
<tr>
<td>18</td>
<td>FSG</td>
</tr>
<tr>
<td>19</td>
<td>ABS</td>
</tr>
<tr>
<td>20</td>
<td>GFS</td>
</tr>
<tr>
<td>21</td>
<td>W-S</td>
</tr>
<tr>
<td>22</td>
<td>DMP</td>
</tr>
<tr>
<td>23</td>
<td>M-S</td>
</tr>
<tr>
<td>24</td>
<td>GAL</td>
</tr>
</tbody>
</table>

Continued...
Comparative study of relative bioavailability between two formulations containing Clozapine 100 mg in patients with schizophrenia

■ evaluation of the bioequivalence average: (i) for ASCτ, Cmin_ss, and Cmax_ss from confidence intervals, and unilateral tests of Schuirmann for differences between averages. The Equivtest® 3.0, Phoenix WinNonLin®, and Excel® spreadsheet programs were used.

RESULTS

In bioequivalence studies on the state of equilibrium, the variables of interest (Cmax_ss, ASCτ) usually follow the log-normal distribution in which case the logarithmic transformation of concentrations should be used. The criteria typically used to declare bioequivalence is that the 90% confidence interval for the ratio of averages is within the interval (0.80; 1.25) or, equivalently, that the 90% confidence interval for the difference between averages on the logarithmic scale (base) is within the interval (-0.2231; 0.2231).11,13

Therefore, the pharmacokinetic measurements used were: minimum drug concentration at the state of equilibrium (Cmin_ss), peak concentration at the state of equilibrium (Cmax_ss), and area under the curve for drug plasma concentration during the state of equilibrium (ASCτ). The average differences (± SD) between reference and testing drugs were: 0.1615 ± 0.3404 (ng/mL); -0.0969 ± 0.4131 (ng/mL), and 0.9143 ± 3.9941 (ng*h/mL). The confidence intervals for average bioequivalence met the limits determined by the National Health Surveillance Agency (ANVISA) of 80 to 125%.

Figure 1 presents the average plasma concentration profiles over time of reference and testing drugs. It may be noted that the curves of the testing (Lifal-Clozapina®) and reference (Clozaril®) drugs are practically overlapping.

Blood samples were collected after 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hours of drug administration. The sequence assigned to each volunteer during the study periods was determined by a randomized list automatically generated.

Blood samples collected at the assigned times were immediately centrifuged. Plasma was divided into two aliquots and stored at 20 °C until analysis. The analytical method for the quantification of Clozapine in the plasma presented adequate specificity, recovery, linearity, quantification limit, precision, and accuracy.

STATISTICAL ANALYSIS

The statistical methodology consisted of the following steps:
- Descriptive analysis of the main pharmacokinetic measurements (ASCτ: area under the curve for drug plasma concentration during the state of equilibrium, Cmax_ss: peak of maximum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the highest observed concentration, Cmin_ss: minimum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the lowest concentration observed, Cav: average of concentrations at equilibrium (ASCτ/τ), degree of fluctuation in the state of equilibrium: GF = (Cmax_ss - Cmin_ss)/(Cav x 100), Swing: (Cmax_ss - Cmin_ss)/Cmin_ss) both individually and as a group of volunteers for each drug in each period;
- analysis of variance for the evaluation of effects of period and sequence;
- Analysis of possible fixed and residual effects is made on the basis of the analysis of variance (ANOVA) for the Cmax_SS, ASCτ, and Cmin_SS data.
- Evaluation of the bioequivalence average: (i) for ASCτ, Cmin_ss, and Cmax_ss from confidence intervals, and unilateral tests of Schuirmann for differences between averages. The Equivtest® 3.0, Phoenix WinNonLin®, and Excel® spreadsheet programs were used.

Blood samples were collected after 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hours of drug administration. The sequence assigned to each volunteer during the study periods was determined by a randomized list automatically generated.

Blood samples collected at the assigned times were immediately centrifuged. Plasma was divided into two aliquots and stored at 20 °C until analysis. The analytical method for the quantification of Clozapine in the plasma presented adequate specificity, recovery, linearity, quantification limit, precision, and accuracy.

Blood samples were collected after 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hours of drug administration. The sequence assigned to each volunteer during the study periods was determined by a randomized list automatically generated.

Blood samples collected at the assigned times were immediately centrifuged. Plasma was divided into two aliquots and stored at 20 °C until analysis. The analytical method for the quantification of Clozapine in the plasma presented adequate specificity, recovery, linearity, quantification limit, precision, and accuracy.

STATISTICAL ANALYSIS

The statistical methodology consisted of the following steps:
- Descriptive analysis of the main pharmacokinetic measurements (ASCτ: area under the curve for drug plasma concentration during the state of equilibrium, Cmax_SS: peak of maximum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the highest observed concentration, Cmin_SS: minimum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the lowest concentration observed, Cav: average of concentrations at equilibrium (ASCτ/τ), degree of fluctuation in the state of equilibrium: GF = (Cmax_SS - Cmin_SS)/(Cav x 100), Swing: (Cmax_SS - Cmin_SS)/Cmin_SS) both individually and as a group of volunteers for each drug in each period;
- analysis of variance for the evaluation of effects of period and sequence;
- Analysis of possible fixed and residual effects is made on the basis of the analysis of variance (ANOVA) for the Cmax_SS, ASCτ, and Cmin_SS data.
- Evaluation of the bioequivalence average: (i) for ASCτ, Cmin_SS, and Cmax_SS from confidence intervals, and unilateral tests of Schuirmann for differences between averages. The Equivtest® 3.0, Phoenix WinNonLin®, and Excel® spreadsheet programs were used.

Blood samples were collected after 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hours of drug administration. The sequence assigned to each volunteer during the study periods was determined by a randomized list automatically generated.

Blood samples collected at the assigned times were immediately centrifuged. Plasma was divided into two aliquots and stored at 20 °C until analysis. The analytical method for the quantification of Clozapine in the plasma presented adequate specificity, recovery, linearity, quantification limit, precision, and accuracy.

STATISTICAL ANALYSIS

The statistical methodology consisted of the following steps:
- Descriptive analysis of the main pharmacokinetic measurements (ASCτ: area under the curve for drug plasma concentration during the state of equilibrium, Cmax_SS: peak of maximum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the highest observed concentration, Cmin_SS: minimum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the lowest concentration observed, Cav: average of concentrations at equilibrium (ASCτ/τ), degree of fluctuation in the state of equilibrium: GF = (Cmax_SS - Cmin_SS)/(Cav x 100), Swing: (Cmax_SS - Cmin_SS)/Cmin_SS) both individually and as a group of volunteers for each drug in each period;
- analysis of variance for the evaluation of effects of period and sequence;
- Analysis of possible fixed and residual effects is made on the basis of the analysis of variance (ANOVA) for the Cmax_SS, ASCτ, and Cmin_SS data.
- Evaluation of the bioequivalence average: (i) for ASCτ, Cmin_SS, and Cmax_SS from confidence intervals, and unilateral tests of Schuirmann for differences between averages. The Equivtest® 3.0, Phoenix WinNonLin®, and Excel® spreadsheet programs were used.

Blood samples were collected after 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hours of drug administration. The sequence assigned to each volunteer during the study periods was determined by a randomized list automatically generated.

Blood samples collected at the assigned times were immediately centrifuged. Plasma was divided into two aliquots and stored at 20 °C until analysis. The analytical method for the quantification of Clozapine in the plasma presented adequate specificity, recovery, linearity, quantification limit, precision, and accuracy.

STATISTICAL ANALYSIS

The statistical methodology consisted of the following steps:
- Descriptive analysis of the main pharmacokinetic measurements (ASCτ: area under the curve for drug plasma concentration during the state of equilibrium, Cmax_SS: peak of maximum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the highest observed concentration, Cmin_SS: minimum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the lowest concentration observed, Cav: average of concentrations at equilibrium (ASCτ/τ), degree of fluctuation in the state of equilibrium: GF = (Cmax_SS - Cmin_SS)/(Cav x 100), Swing: (Cmax_SS - Cmin_SS)/Cmin_SS) both individually and as a group of volunteers for each drug in each period;
- analysis of variance for the evaluation of effects of period and sequence;
- Analysis of possible fixed and residual effects is made on the basis of the analysis of variance (ANOVA) for the Cmax_SS, ASCτ, and Cmin_SS data.
- Evaluation of the bioequivalence average: (i) for ASCτ, Cmin_SS, and Cmax_SS from confidence intervals, and unilateral tests of Schuirmann for differences between averages. The Equivtest® 3.0, Phoenix WinNonLin®, and Excel® spreadsheet programs were used.

Blood samples were collected after 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 hours of drug administration. The sequence assigned to each volunteer during the study periods was determined by a randomized list automatically generated.

Blood samples collected at the assigned times were immediately centrifuged. Plasma was divided into two aliquots and stored at 20 °C until analysis. The analytical method for the quantification of Clozapine in the plasma presented adequate specificity, recovery, linearity, quantification limit, precision, and accuracy.

STATISTICAL ANALYSIS

The statistical methodology consisted of the following steps:
- Descriptive analysis of the main pharmacokinetic measurements (ASCτ: area under the curve for drug plasma concentration during the state of equilibrium, Cmax_SS: peak of maximum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the highest observed concentration, Cmin_SS: minimum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the lowest concentration observed, Cav: average of concentrations at equilibrium (ASCτ/τ), degree of fluctuation in the state of equilibrium: GF = (Cmax_SS - Cmin_SS)/(Cav x 100), Swing: (Cmax_SS - Cmin_SS)/Cmin_SS) both individually and as a group of volunteers for each drug in each period;
Comparative study of relative bioavailability between two formulations containing Clozapine 100 mg in patients with schizophrenia

**DISCUSSION**

To achieve a statistical variability as low as possible, we selected volunteers with physical and clinical characteristics that were mostly similar.

On the basis of blood collections before hospitalization in each period, it was concluded that the state of equilibrium of serum levels was achieved in the two study periods.

Because the confidence intervals for the averages ratios of $C_{\text{max,ss}}$, $ASC_\tau$, and $(C)_{\text{min,ss}}$ are completely within the 80-125% interval according to the requirement from ANVISA, it can be concluded that there is an average bioequivalence between the testing (LifalClozapina® 100 mg) and the reference (Leponex® 100 mg) drug.

The $2 \times 2$ crossover planning was adequate for the type of drug, there was no violation of assumptions in the statistical methods used, and the number of volunteer participants, in addition to meeting the determination by ANVISA for bioequivalence studies, provided a quite high statistical power.

The sample planning and experimental conducts were carried out correctly and efficiently. There was no difference in adverse/side effects due to the exchange between testing and reference drugs. The difficulties encountered were related to the population in the study, which should be composed of individuals with schizophrenia. In addition, this group should undergo a long period of study to ensure reaching the state of equilibrium. All was effectively managed by the multidisciplinary team from CEBIO, leading to a study that reached the proposed objective without, however, producing negative effects on the studied population.

Finally, it is important to emphasize the importance of this study which, in verifying the equivalence between these two formulations, made the availability of affordable Clozapine in the market possible. Hence, the study ensured that more people could take advantage of this treatment, so far limited to those who had purchasing power to afford the treatment, providing easier access to this drug that is so important for the control of schizophrenia.

**REFERENCES**

Comparative study of relative bioavailability between two formulations containing Clozapine 100 mg in patients with schizophrenia


