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

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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 à 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*

considerados bioequivalentes. Assim, produtos teste e referência são considerados intercambiáveis.

Palavras-chave: Disponibilidade Biológica; Equivalência Terapêutica; Clozapina; Farmacocinética; Esquizofrenia.

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 n° 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.[1-7]

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

volunteers. The volunteers received multiple doses of the testing and reference drugs until reaching the equilibrium state.

The population selected to participate in the study consisted of 40 volunteers, all males, and already users of Clozapine. The age range of the volunteers was from 18 to 55 years old. The selection process followed a rigorous clinical, cardiological, and psychiatric evaluation. The study was finalized with 35 volunteers because five dropped out of participation immediately before the beginning of drug administration.

Patients received random doses of 100 mg (or multiple of 100 mg) according to the previous dose used by the patient, twice daily of the reference/testing formulation of Clozapine (LifalClozapina®, testing formulation/Leponex®, reference formulation). The administration was conducted during fasting, at around 7 am and before dinner at 7 pm for 10 days before the first study period. To ensure that volunteers took the drug correctly during the domicile period, a nursing team was present at the domicile of each patient during administration. The administration of drugs followed a scheme, as exemplified below:

Table 1 - Drug Administration and sample collection

Days	Procedures
1st to T	Testing/Reference Drugs
T to 9th	Testing/Reference Drugs (blood sample to evaluate equilibrium and hospitalization on the 9th)
10th	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)
11th	Exchanging drugs (resumed drug administration at home)
12th - 13th	Medicamento Teste/ referência
14th – 16th	Testing/Reference Drugs (blood sample to evaluate equilibrium and hospitalization on the 16th)
17th	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)

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

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.

Table 2 - Dose used for each volunteer

Volunteers			Dose
ID	Initials	Gender	Every 12 hours
1	FCM	M	200 mg
2	CAT	M	100 mg
3	WSD	M	300 mg
4	PES	M	200 mg
5	ITC	M	100 mg
6	OAL	M	200 mg
7	FFP	M	200 mg
8	PHR	M	200 mg
9	EFP	M	200 mg
10	RRO	M	100 mg
11	IMS	M	200 mg
12	MLC	M	200 mg
13	JVO	M	300 mg
14	MAF	M	200 mg
15	RBJ	M	300 mg
16	MPR	M	200 mg
17	EJR	M	200 mg
18	FSG	M	300 mg
19	ABS	M	100 mg
20	GFS	M	300 mg
21	W-S	M	200 mg
22	DMP	M	300 mg
23	M-S	M	100 mg
24	GAL	M	300 mg

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Table 2 - Dose used for each volunteer

Volunteers			Dose
ID	Initials	Gender	Every 12 hours
25	RPE	M	100 mg
26	MVS	M	300 mg
27	PID	M	200 mg
28	WAS	M	300 mg
29	GDC	M	200 mg
30	JSM	M	200 mg
31	JNS	M	200 mg
32	VSV	M	100 mg
33	LMS	M	100 mg
34	MSC	M	200 mg
35	DRL	M	100 mg

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, C_{max_ss}: peak of maximum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the highest observed concentration, C_{min_ss}: minimum drug concentration and/or metabolite at equilibrium obtained directly without data interpolation, that is, the lowest concentration observed, C_{av}: average of concentrations at equilibrium (ASCτ/τ), degree of fluctuation in the state of equilibrium: GF = $(C_{max\_ss} - C_{min\_ss})/(C_{av}$ x 100), Swing: $(C_{max\_ss} - C_{min\_ss})/C_{min\_ss})$ 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;

■ evaluation of the bioequivalence average: (i) for ASCτ, C_{min_ss}, and C_{max_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 (C_{max_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 (C_{min_ss}), peak concentration at the state of equilibrium (C_{max_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.

The pharmacokinetic measurements calculated for the testing and reference formulations are presented in Table 3.

The verification of possible residual and fixed effects is made on the basis of the analysis of variance (ANOVA) for the C_{max_ss}, ASC τ, and $(C)_{min\_ss}$ data. The significance level of 10% was adopted for the effect of sequence and 5% for the effects of formulation and period. The results for the logarithmic scale data for the three pharmacokinetic measurements showed that there is no statistically significant difference in sequence, formulation, and period effects. However, inter-individual variation in these measurements was observed.
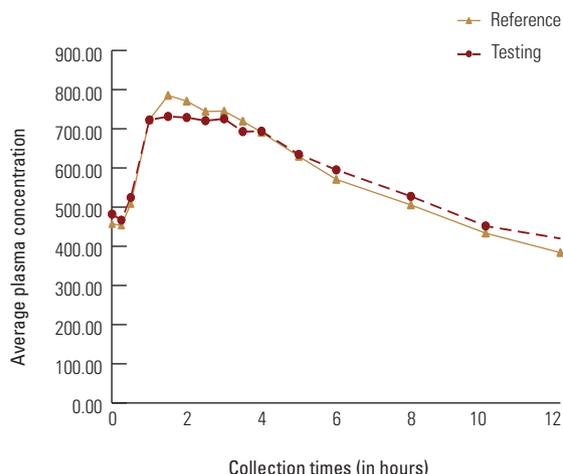
Figure 1 - Plasma concentration curves (ng/mL); average of the two drugs (reference and testing) based on 35 volunteers.

Table 3 - Pharmacokinetic measurements of testing and reference drugs (n = 35)

Pharmacokinetic measurements	Testing drug	Reference drug
ASC τ (ng.h/mL)	36.70 ± 19.53	35.79 ± 15.54
Cmax_ss (ng/mL)	4.32 ± 2.03	4.42 ± 1.62
Cmin_ss (ng/mL)	2.15 ± 1.37	1.99 ± 1.03
Tmax (h)	2. 13 ± 1.30	1.90 ± 1.03
CAV	1.53 ± 0.81	1.49 ± 0.65
Fluctuation (%)	0.016 ± 0.007	0.018 ± 0.007
Swing	1.30 ± 0.77	1.56 ± 1.07

Average ± standard deviation.

The 90% confidence interval for ASCτ was from 0.9340 to 1.0640; for C_{max_ss} from 0.8806 to 1.0238; and C_{min_ss} from 0.9679 to 1.1283. Based on these results, it was observed that the confidence intervals for averages in the three pharmacokinetic measurements are completely within the interval from 0.80 to 1.25.

The following adverse events were reported: out of the 35 volunteers, 10 showed some event; some exhibited more than one episode. The following were detected: one volunteer with fever, two with vomiting, two with headache, and two with anxiety. There was also an episode of insomnia, one of lipotimia status, sweating, and visual hallucination. All events were controlled during hospitalization with no subsequent complaints. In addition, the episodes were not assigned to the drug under study; their origins are based on the prior disease of volunteers and external causes.

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_{max_ss}, ASC τ, and $(C)_{min\_ss}$ 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 x 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.

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