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BIOEQUIVALENCE OF LISDEXAMFETAMINE DIMESYLATE HARD CAPSULES FORMULATIONS

BIOEQUIVALÊNCIA DE FORMULAÇÕES DE CÁPSULAS DURAS DE DIMESILATO DE LISDEXANFETAMINA

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ABSTRACT. Lisdexamfetamine (LDX), an inactive prodrug of dexamphetamine, is used as a second-line treatment for attention deficit hyperactivity disorder (ADHD) and for moderate to severe binge eating disorder (BED). The objective of this study is to evaluate the bioequivalence of two LDX formulations, including the reference product manufactured by Patheon Pharmaceuticals (Venvanse® 70 mg) versus a formulation manufactured by Eurofarma Laboratórios S/A. A randomized, crossover, open-label study was conducted with two treatments, two periods, two sequences, and 48 healthy participants of both sexes who received oral administration of the medications. A total of 24 blood samples were collected from each participant, from T0 (before taking the medication) to 10 hours later. The plasma concentration of LDX was quantified using liquid chromatography coupled with mass spectrometry (LC-MS/MS). Both formulations were well tolerated, and no serious adverse events were reported. Cmax and AUC0-t were compared: the ratio between the test and reference formulations for Cmax was 106.22% with a 95% confidence interval (CI) of 97.72% - 116.66% and a power of 98.75%. The ratio between the test and reference formulations for AUC0-t was 106.28%, CI (100.68% - 112.19%), with a power of 100.00%. The ratio between the test and reference formulations for AUC0-inf was 106.29%, CI (100.71% - 112.17%), with a power of 100.00%. The formulations were shown to be statistically bioequivalent in terms of their rate and extent of absorption, based on criteria established by the Brazilian Health Regulatory Agency (ANVISA).

Keywords: Attention deficit disorder; hyperactive; lisdexamfetamine dimesylate; bioequivalence; biological availability; pharmacokinetics.

RESUMO. A lisdexanfetamina (LDX), pró-fármaco inativo da dexanfetamina, é usada como tratamento de segunda linha para transtorno de déficit de atenção/hiperatividade (TDAH) e para transtorno de compulsão alimentar periódica (TCAP) moderado a grave. O objetivo deste trabalho é avaliar a bioequivalência de duas formulações de LDX, incluindo o produto de referência fabricado pela Patheon Pharmaceuticals (Venvanse® 70mg) versus uma formulação fabricada pela Eurofarma Laboratórios S/A. Foi realizado estudo randomizado, cruzado e aberto, com dois tratamentos, dois períodos, duas sequências e 48 participantes saudáveis de

ambos os sexos receberam a administração oral dos medicamentos. Foram coletadas 24 amostras de sangue de cada participante desde o T0 (antes de tomar o medicamento) até 10 horas depois. A concentração plasmática de LDX foi quantificada por cromatografia líquida acoplada à espectrometria de massa (LC-MS/MS). Ambas as formulações foram bem toleradas e nenhum evento adverso grave foi relatado. A Cmax e a AUC0-t foram comparadas: a taxa entre as formulações de teste e de referência para a Cmax foi de 106,22% com IC (97,72% - 116,66%) e potência de 98,75%. A taxa entre as formulações de teste e de referência para AUC0-t foi de 106,28% CI (100,68% - 112,19%) e potência de 100,00%. A taxa entre as formulações teste e referência para AUC0-inf foi de 106,29% com IC (100,71% - 112,17%) e poder de 100,00%. As formulações demonstraram ser estatisticamente bioequivalentes, de acordo com sua taxa e extensão de absorção, com base em critérios da Agência Nacional de Vigilância Sanitária (ANVISA).

Palavras-chave: Transtorno de déficit de atenção; hiperatividade; dimesilato de Lisdexanfetamina; bioequivalência; disponibilidade biológica; farmacocinética.

INTRODUCION

Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder estimated to affect 8-10% of children and teenagers, and 2.5-4% of adults, worldwide. ADHD core symptoms are inattention, impulsivity, and hyperactivity. The diagnosis of ADHD is made through clinical evaluation, utilizing primarily diagnostic frameworks such as the Diagnostic and Statistical Manual of Psychiatric Disorders (DSM-5: American Psychiatric Association) and the International Classification of Diseases 11th revision (ICD-11: World Health Organization) (CASTELLS et al., 2018).

Stimulants, available as long-acting or short-acting medications, are frequently advised as a component of a comprehensive multimodal treatment strategy for ADHD, which may additionally encompass behavioral, psychoeducational, and psychological interventions. Apart from decreasing or eliminating the necessity for multiple daily administrations, the proposed advantages of extended-release stimulants also involve enhanced adherence and reduced potential for abuse when compared to their short-acting counterparts (CASTELLS et al., 2018).

LDX is an inactive prodrug of dexamfetamine (KRISHNAN et al., 2008; HUTSON et al., 2014), that is converted to the therapeutically-active metabolite, d-amphetamine. Following oral administration, lisdexamfetamine LDX is rapidly taken up from the small intestine by active carrier-mediated transport, probably via peptide transporter. Once in the blood, the prodrug is hydrolyzed in erythrocyte cytosol by an unknown aminopeptidase, thus releasing L-lysine (a naturally occurring essential amino acid) and the active dexamfetamine, with an estimated conversion efficacy of 98% (HUTSON et al., 2014; PENNICK, 2010).

The dexamfetamine generated crosses the blood-brain barrier to access binding sites in the central nervous system and to exert therapeutic effects by increasing noradrenergic and dopaminergic neurotransmission. Literary reviews carried out suggest that: “lisdexamfetamine has the best benefit risk balance and has promising potential for treating children and adolescents with ADHD” (PENNICK, 2010).

LDX is the only prodrug available for treatment of ADHD, and its patent expiration can improve accessibility and affordability for ADHD patients looking for treatment options, as new generic versions get their approval from regulatory agencies.

The objective of this study was to obtain relevant pharmacokinetic parameters for statistical comparison, aiming to establish evidence of bioequivalence between a new formulation of LDX capsules developed by Eurofarma Laboratórios S/A and the currently referenced drug, Venvanse® 70 mg formulation, produced by Patheon Pharmaceuticals and imported by Takeda Pharma Ltda. Pharmacokinetic parameters were assessed directly from the plasma concentration of LDX following drug administration, based on the application of a non-compartmental model suitable for evaluating concentrations after a single orally administered dose.

METHODS

Study design

This was an open, randomized, crossover study, under fast condition, with two treatments, two periods and two sequences of reference medicine vs. Test drug (RT sequence) or test drug vs. Reference medicine (TR sequence).

Subjects and samples

Forty-eight research participants (male and woman subjects) were selected in this trial. All participants were 18 to 50 years old and had to have a body mass index (BMI) between 18.5 and 29.9 kg/m². Subjects who participated in any other clinical trial in the last 6 months prior to the initiation of the study were also excluded, as well as the ones with history of drug abuse and history of allergy to LDX. Other exclusion criteria were the use of any medication within 14 days before the study, positive result for hepatitis B, C, HIV or any clinically significant alteration of laboratory exams. Research participants who presented liver enzyme values varying ±10% from the value considered normal were not accepted into the study. In addition, volunteers that ingested foods or drinks containing grapefruit, foods or drinks containing poppy seeds (breads, cakes and yogurts with poppy seeds, etc.) during the 14 days preceding the study and ingested cabbage or broccoli (known inhibitors and inducers of the CYP450 system), dried plums, raspberries and grapefruit were not selected.

All participants signed an Informed Consent Form, which was approved by the Ethics Committee of Sao Francisco University (CAAE: 64796222.6.0000.5514 approved 11/11/22), along with the clinical protocol 2022. Further, all procedures of the study were conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines and local ethics regulations. The study was conducted according to CNSP 4522.

The formulations were administered orally in a single dose of each formulation, followed by blood sampling until 10 hours after administration, per period. Trial subjects remained under fasting conditions for 4 hours for solid meals and 2 hours for water after administration and they remained confined for approximately 48 hours (two periods total duration).

Trial subjects received one orally dose of either test or reference formulations, according to a randomization list. Blood samples of 8.5 mL were collected through a catheter in the forearm vein. A total of 25 samples were collected in a tube containing ethylenediamine tetraacetic acid (EDTA). Sampling times were 0:00, 0:10, 0:20, 0:30, 0:40, 0:50, 1:00, 1:10, 1:20, 1:30, 1:40, 1:50, 2:00, 2:15, 2:30, 2:45, 3:00, 3:20, 3:40, 4:00, 5:00, 6:00, 7:00, 8:00 and 10:00 hours after administration. Samples were centrifuged (3.000 rpm 10 minutes) and the

plasma obtained from this process was separated into two aliquots kept in cryogenic tubes and stored at -20°C.

Sample extraction and LC-MS/MS analysis

Plasma samples (200 µL) were doped with 50 µL of the internal standard Lisdexamfetamine-D4 (LDX-D4) at 20 ng/mL. Aftward, 25 µL of Ammonium Hydroxide (30% v/v) were added, followed by 4 mL of a solution of Diethyl Ether:Dichloromethane (70:30; % v/v). Samples were vortexed and centrifuged. After that, it is stored in an ultrafreezer. The supernatant was poured into a glass test tube and dried under nitrogen flow at 40 ºC. Prior to analysis, samples were resuspended with 400 µL of mixture of water with 5 mM Ammonium Formate and 22.5 mM Formic Acid, and Acetonitrile (80/20; % v/v).

Determination of the plasmatic concentrations of LDX were conducted in an HPLC coupled to a mass spectrometer from Waters (Waters Corporation, Milford, CT, USA). An Zorbax Eclipse Plus C18 (3,5 µm, 100x4.6 mm) column from Agilent was used for LC separation. Separation was performed using an isocratic elution of Water + 5 mM Ammonium Formate + 22.5 mM Formic Acid /Acetonitrile (85/15; % v/v), at a flow rate of 0.800 mL/min. Total running time was 5 minutes. The mass spectrometer was operated in position electrospray ionization mode (ESI+). Data was acquired using Multiple Reaction Monitoring (MRM) mode by monitoring the following precursor>fragment transitions: ions with a mass-to charge (m/z) ratio of 264,0 > 84,0 for LDX, and m/z 268,0 > 88,0 for LDX-D4. Other parameters were as follows: The temperature of the source was 600° for source temperature, with Ion Spray Voltage of -4,5 kV for capillary, 4500 V and medium collision gas flow. Collision energy (CE) was set to -20V for Lisdexamfetamine LDX and -17V for Lisdexamfetamine LDX-D4.

Concentrations of the analyte in the samples were determined from calibration curves, defined by a linear regression model, which points were obtained from blank human plasma, contaminated with known concentrations of the analyte (LDX) and of the internal standard (LDX- D4), prepared as described for test samples. For quantification, the ratio of the areas of the LDX/LDX-D4 peaks was used. The methodology for the current study was validated for the range of 0.200 to 160.000 ng/mL of LDX. Method validation included: selectivity, linearity, precision and accuracy of the lower limit of quantification, precision and accuracy of the quality control samples and for the control of dilution, matrix effect, recovery test and residual effect. Stability tests for post-processing, short and long duration, freeze and thaw, analyte in solution and internal standard in solution were also performed.

Data analysis

The statistical analysis of the data was done with Phoenix/WinNonlinTM version 8.2 (CERTARA, 2019), Microsoft® Excel®, version 97-2003, Microsoft® Word® version 97-2003. All calculations of pharmacokinetic parameters were done with plasma concentration obtained from analytical determinations for each collected sample. Bioequivalence assessment was performed using 90% Confidence Intervals (based on the Schuirmann test (SCHUIRMANN, 1987) for pharmacokinetic parameters transformed into natural logarithms (Ln). The construction of these intervals is based on the residual mean square of the Analysis of Variance (ANOVA), which was constructed according to the 2 x 2 Crossover design and contains the effects of sequence (fixed effect), subject within the sequence (random effect), formulation (fixed effect) and period (fixed effect). For the formulations to be considered

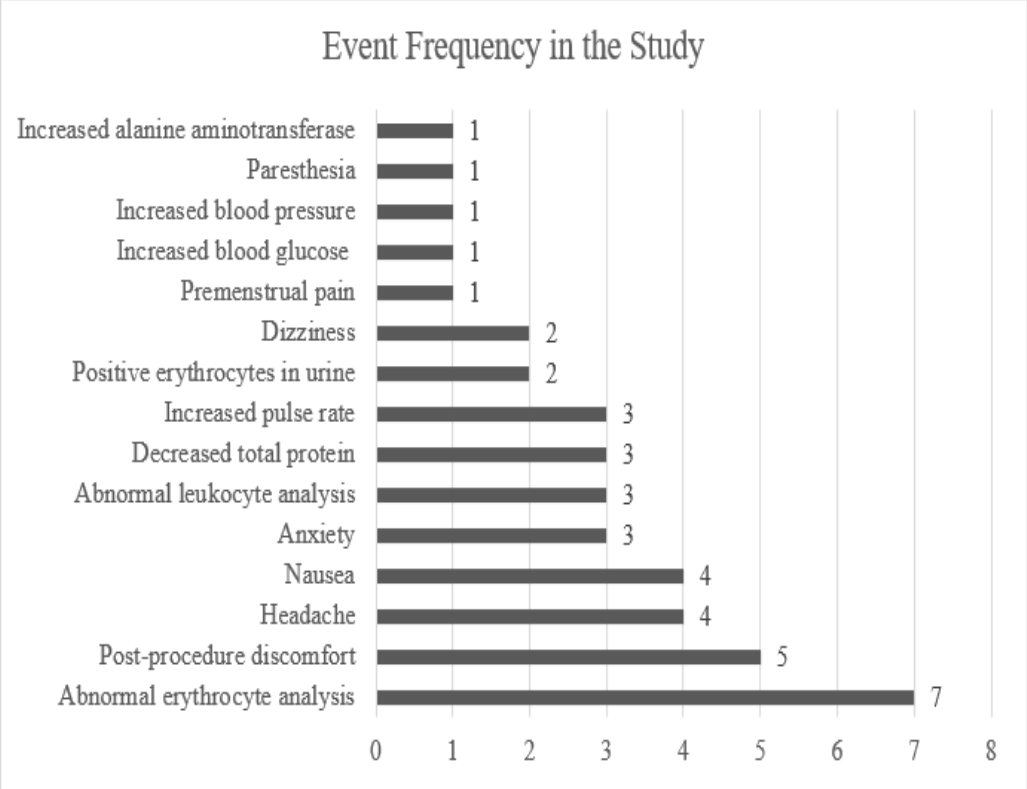
bioequivalent, the confidence interval must be contained between 80 and 125%, according ANVISA parameters (BRASIL, 2006).

RESULTS AND DISCUSSION

A total of 46 subjects ended the study. Clinical exams post-study did not show modifications in the general health well-being of the participants that could be attributed to the study products. Both formulations were well tolerated in the administered dose and no serious adverse events were reported

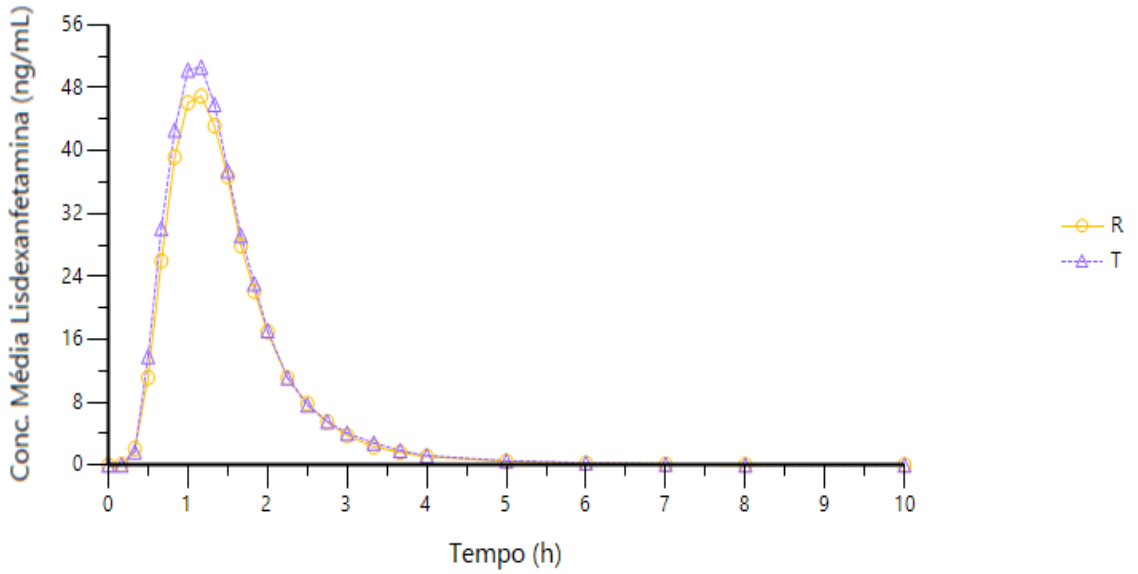
Some adverse events possibly related to the treatment were reported during the study, such as: anxiety, headache and nausea. The most common adverse events not related to the treatment were alterations of post-study laboratory exams. All adverse events were resolved without sequels and were described in Figure 1.

Figure 1. Frequency of adverse events reported during the study. Note: Adverse events according to the Medical Dictionary for Regulatory Activities (MedDRA, version 26.1[8]) primary system organ class and preferred term (MedDRA, 2023).



The mean plasmatic concentration of LDX after the administration can be found in Figure 2.

Figure 2. Comparison curve between test x reference of mean plasmatic concentrations of LDX vs time. Legend: Test drug (T); Reference medicine (R).



The present study reveals that both formulations have similar concentration curves. Considering p-values shown in Table 1, above, it is possible to conclude that sequence effect is not significant (p-value = 0.248) for Cmax, is not significant for AUC0-t (p -value = 0.461), considering a significance level of 10%.

Table 1. Statistical results of the pharmacokinetics parameters Cmax, AUC 0-t and AUC0-inf.

Parameter	N	T/R Ratio %	Confidence Interval (90%)	CV (intra) %	Power %	P Value (sequence effect)
C_{max}	46	106,222	(96,721 – 116,656)	27,230	98,752	0,248
AUC_{0-t}	46	106,280	(100,679 – 112,191)	15,543	100,000	0,461
AUC_{0-inf}	46	106,285	(100,710 – 112,168)	15,469	100,000	0,449

The period effect is not significant (p-value = 0.254) for Cmax and it is not significant for ASC0-t (p-value = 0.054), at a significance level of 5%. The ANOVA test did not show significant statistical difference between both formulations for product effect and sequence effect for the parameters Cmax, Tmax. No significant differences were observed between the test and reference formulations for AUC 0-t and AUC0-inf.

Considering that there are currently only 7 valid registrations for the molecule in Brazil and the high cost of treatment, this article presents the inclusion of another manufacturer of the generic/similar medicine, bringing the possibility of promoting cost reduction for patients.

It is important to highlight that generics law in Brazil (Law 9787/1999) was an important milestone for Brazilian public health, allowing the population greater access to medicines, as well as regulating and enabling the production and commercialization of medicines with expired patents, by the pharmaceutical industry.

CONCLUSION

The test drug lisdexamfetamine dimesylate, 70 mg hard capsule from Eurofarma Laboratórios S/A is bioequivalent to reference medicine Venvanse®, 70 mg hard capsule, from Patheon Pharmaceuticals Inc. and imported by Takeda Pharma Ltda, once the rate and extension of absorption were considered as required by ANVISA (Brazilian National Health Surveillance Agency). The rates of means of minimum squares and confidence intervals of 90% derived from the analysis of pharmacokinetic measurements (log-transformed) of AUC0-t, ASC0- inf (100.679 – 112.191%) and Cmax (96.721 – 116.656%) for Lisdexamfetamine are within the limit of 80-125%.

The present study is of great relevance to Brazilian society, as it will collaborate with the supply of the market, enabling possible cost reduction in treatments for patients with ADHD and consequently allowing greater access to the medication. The bioequivalence study was sponsored by Eurofarma Laboratórios S/A, and clinical step conducted by UNIFAG Research Center and an analytical step performed by Magabi Clinical Research.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest in this study or publishing work.

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