

Randomized, single-dose, two-period, two-sequence crossover bioequivalence study evaluating two oral formulations of lamotrigine in healthy volunteers

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ABSTRACT

Background: Lamotrigine is an anti-epileptic medicine used to treat epilepsy and bipolar disorder. The mechanism of action is to block voltage activated sodium channels. The aim of this study was to evaluate the bioequivalence of 2 oral formulations of lamotrigine 25 mg in healthy volunteers.

Methods: A single-dose, two-period, randomized crossover study design in - healthy Indian adult volunteers was conducted at Amaris Clinical, a division of Caplin Point Laboratories Ltd., Chennai. A validated high performance liquid chromatography in conjunction with mass spectrometry was used. Lamotrigine concentration in plasma. Adverse events were determined by measuring vital functions after dosing. A total of 24 subjects were included.

Results: The mean and 90% confidence intervals of the test / reference ratios for these parameters were as follows: The mean C_{max} and T_{max} of the test were 758.606 (157.453) ng / ml and 1.17 (0.50-5.00) hours, respectively. The mean C_{max} and T_{max} of the reference were 775.993 (151.654) ng / ml or 0.88 (0.25-4.00) hours. The mean AUC₀₋₇₂ was 24142.031±3641.691 (ng.hr/mL) for the test formulation and 24202.099±3742.957 (ng. h / ml) for the reference formulation. The mean test / reference ratios for C_{max} and AUC₀₋₇₂ were 97.92 and 99.82 respectively. The 90% parametric CIs for C_{max} and AUC₀₋₇₂ were 90.17-105.68% or 97.87-101.81%.

Conclusions: The 90% confidence intervals ranged from 80-125% and it was concluded that the test product was bioequivalent to the reference product in these healthy adult male volunteers.

Keywords: Bioequivalent, Lamotrigine, Pharmacokinetics, Reference, Test

INTRODUCTION

Epilepsy is considered the most common neurological disorder and is generally controlled. However, the main obstacle is that it cannot be cured with medication. Basically, spontaneous electrical activity occurs in the brain. Much research is being done to find new drugs to cure, but any of the anticonvulsants currently available can inhibit the development or spread of this abnormal spontaneous electrical activity. Available anticonvulsants

are poorly effective in epilepsy syndromes and have a relatively high frequency of serious side effects. The newer anticonvulsants are viewed only as supplements or alternatives to replacing or combining with already available epilepsy drugs, with an emphasis on improving patient safety or better seizure control. Lamotrigine was approved by the Food and Drug Administration (FDA) for the treatment of epilepsy in 1994 (FDA).¹ The main treatment is recommended as monotherapy for partial seizures and type I bipolar disorder.² It is also an additional

option for treating partial seizures, generalized seizures of Lennox-Gastaut syndrome, and tonic clonic seizures with primary generalization.³ Lamotrigine is a voltage-dependent and usage-dependent blocker of voltage-gated sodium channels. It inhibits the sustained repeated activation of neurons and the release of glutamate (neurotransmitter that plays a key role in the development of epileptic seizures).⁴ These effects likely contribute to the anticonvulsant properties of lamotrigine. Lamotrigine pharmacokinetics are characterized by rapid and complete absorption from the gut without significant first-pass metabolism. Peak plasma concentrations occur approximately 2.5 hours after oral administration of lamotrigine. The volume of distribution is 0.92 to 1.22 l/kg.⁵ Apparent plasma clearance in healthy volunteers is approximately 30 ml/min. Lamotrigine clearance is primarily metabolic with subsequent elimination of glucuronide-conjugated material in the urine. Less than 10% is excreted unchanged in the urine.⁶ The shelf life of lamotrigine ranges from 22.8 to 37.4 hours.^{4,5} The therapeutic range for seizure control is 1 to 4 mg/L, although this is not well documented.^{9,10} This study was designed to assess the bioequivalence of lamotrigine tablets (2×25 mg) and the reference product LAMICTAL® after oral administration of a single dose.

METHODS

Lamotrigine 25 mg, reference product and test product were considered. Before the bioequivalence study, the products were tested for potency, dissolution and dosage uniformity through analytical methods used by the manufacturer. The products were judged suitable for the bioequivalence study if the assayed potency of the test product not differ from that of the reference product by more than 5%; the mean percentage dissolved in 30 minutes was not less than 80% and the acceptance value for dosage uniformity of the 10 dosage units is less than or equal to 15%.

Subjects

24 healthy adult human subjects, non-smoking, males and/or non-pregnant, non-lactating female literate volunteers of 18 to 45 years (both years inclusive) with a BMI of 18.50 – 30.00 Kg/m² for males and females were eligible to be enrolled in the study. Inclusion criteria includes healthy volunteers as evaluated by medical history, vitals and general clinical examination, normal or clinically insignificant biochemical, haematological, urine and serology parameters, normal or clinically insignificant ECG and Chest X-ray, negative urine test for drugs of abuse, alcohol breath analysis for both males and females and negative pregnancy tests for females, subjects who are willing to practice acceptable methods of contraception, volunteers who can give written informed consent form and communicate effectively.

Subjects were informed by the investigator about the purposes and risks of the study and a written informed

consent was obtained from all volunteers. They were asked to stop using any concomitant medications, including over-the-counter products, dietary supplements and natural products, two weeks prior to dosing and throughout the end of the study. Subjects were housed in the Clinical pharmacology unit (CPU), checked in at least 12.00 hours before the proposed time of drug administration in each period of the study. Being a fasting study subjects were required to fast for at least 12 hours before each scheduled dosing.

Study design

The protocol and study design were reviewed and approved by the Ethics Committee, registered with the DCGI under rule 122D of drugs and cosmetics Rules 1945. The study was performed in compliance with the principles of the Declaration of Helsinki the guidelines for Good clinical practice.^{7,8}

It is an open label, randomized, two treatment, two sequence, two period, single dose, cross over, bioequivalence study. Study subjects received any one of the investigational product in each period as per the randomization schedule. Minimum 21 days of washout period was maintained between the administrations in each treatment period.

Study drug administration and blood sampling

A single oral dose of test (2×25 mg) (T) or Reference product (2×25 mg) (R) was administered to study subjects in sitting posture at fixed time with 200±02 mL of water in each period. The order of receiving test and reference products will be as per the randomization schedule. This activity will be followed by mouth and hands check of the subjects to assess compliance to dosing. Subjects will remain in an upright position for 04 hours after dosing. During this period, subjects will be seated and may be permitted to walk in an upright position for natural exigencies and study procedures. Clinical confinement was from at least 12.00 hours prior to drug administration and until 48.00 hours post dosing. Total duration of the study was minimum 30 days.

Being a fasting study, subjects were not served breakfast on the day of dosing. Drinking water will not be permitted 01 hour before dosing and until 01 hour post-dose, at all other times drinking water will be permitted ad libitum. The subjects will receive standard food.

Total of 26 samples were collected. First 22 samples, collected in clinic and remaining samples, collected as ambulatory. The pre-dose samples collected within 01 hours prior to drug dosing and the post-dose samples will be collected within 02 minutes of the scheduled time. Ambulatory samples will be collected with the window period of ±02 hours from scheduled time.

Blood samples collected during the study were centrifuged at 4000 rpm for 10 minutes at 4°C. Plasma will be separated into 02 aliquots and stored at about -80°C or colder till analysis. The first aliquot will have 01 mL of plasma and remaining plasma in the second aliquot.

Tolerability

If any Adverse event (AE) or Serious adverse event (SAE) occurs during the study, the causality relationship between the study drug and the event and details of the study drug and concomitant medication were assessed. Adverse events (AE) were collected based on interview and spontaneous reports and recorded on a case-report form. The study physicians/ investigators graded the AEs as mild, (it interferes with daily activities), moderate (it interferes with daily activity but it is still able to do it), or severe (it is disabling and requires medical attention).

Determination of plasma concentrations of lamotrigine

Validated LC-MS/MS methods was used for the estimation of Lamotrigine in plasma. Bio-analytical method validation will be done as per ANVISA's bio-analytical method validation guidance, with evaluation for specificity, sensitivity, precision and accuracy, stability, recovery and dilution integrity. Subject samples will be analyzed using these validated methods. Samples of all the subjects who successfully completed both periods of the study will be considered for analysis.

Pharmacokinetic and statistical analysis

The Pharmacokinetic parameters (C_{max}, AUC_{0-t}, AUC_{0-∞}, T_{max}, T_{1/2}, Kel, V_d, CL and AUC_{0-t} / AUC_{0-∞} × 100) of Lamotrigine from test product as compared to that from reference product will be calculated using non compartmental model of Phoenix® WinNonlin v 8.1. The concentration values which are reported as BLQ will be set to "Zero" for all pharmacokinetic and statistical evaluation. The concentration data of all the subjects who successfully completed both periods of the study will be analyzed. Primary PK parameters: C_{max}, AUC_{0-t} and AUC_{0-∞}. secondary PK parameters: T_{max}, T_{1/2}, Kel, V_d, CL and AUC_{0-t} / AUC_{0-∞} × 100.

Statistical analysis will be performed on the plasma concentration values and pharmacokinetic parameters using SAS® v 9.4. The analysis will include data from subjects who complete all the periods of the study. If there are drop outs, no replacement will be done.

Descriptive analysis of plasma concentration (time point wise and formulation wise) and pharmacokinetic parameters – (C_{max}, AUC_{0-t}, AUC_{0-∞}, T_{max}, T_{1/2}, Kel, V_d, CL and AUC_{0-t} / AUC_{0-∞} × 100) will be reported for test and reference products. The reported parameters will be the mean, minimum, maximum, range, standard deviation, Standard error, geometric mean and the coefficient of variation. The Ln-transformed

pharmacokinetic parameters (C_{max}, AUC_{0-t}, and AUC_{0-∞}) will be analyzed using ANOVA Model with the main effects of treatment, period, and sequence as fixed effects and subjects nested within the sequence as a random effect. A separate ANOVA model will be used to analyze each of the parameters. The sequence effect will be tested at the 0.10 level of significance using the subjects nested within sequence mean square from the ANOVA as the error term. All other main effects will be tested at the 0.05 level of significance against the residual error (mean square error/MSE) from the ANOVA as the error term. The Ln transformed primary PK parameters (C_{max}, AUC_{0-t} and AUC_{0-∞}) will be subjected to ratio analysis. The test / reference ratio will be calculated for log transformed primary PK parameters.

To establish bioequivalence between test and reference products, 90% Confidence Interval (CI) for the ratio (Test/Reference) of Least Square Means of the Ln transformed PK parameters (C_{max}, AUC_{0-t} and AUC_{0-∞}) must fall between 80.00% -125.00% for Lamotrigine. Confidence Interval (CI) values should not be rounded off. Therefore, to pass a CI limit of 80-125, the value should be at least 80.00 % and not more than 125.00%.

RESULTS

A total of 23 subjects were enrolled and 22 subjects completed the study. Thus, samples from 22 subjects were analyzed for determining the concentrations of Lamotrigine in plasma. The drug concentration data of 22 subjects were included for pharmacokinetic analysis of Lamotrigine.

Table 1: Demographic profile of subjects who completed the study (N=22).

Parameter	Mean	SD	Min	Max
Age (years)	32.5	5.21	23	40
Height (m)	167.1	6.324	156.7	179.6
Weight (Kg)	70.00	8.9	51.9	84.45
BMI (Kg/m ²)	25.02	2.603142	18.9	30

Demographic and other baseline characteristics: The mean age, height, weight and BMI of all the subjects who completed the study are presented in (Table 1). All the subjects included in the study were male and Asian.

Pharmacokinetic and statistical analysis

The plasma concentration (ng/mL) for test product and reference product are presented in statistical report. Pharmacokinetic parameters were calculated using Phoenix® WinNonlin® software (version 8.1). The mean, standard deviation, standard error, geometric mean,

coefficient of variation, minimum, median, maximum and range were calculated for C_{max}, AUC₀₋₇₂ and T_{max}.

Table 2: Pharmacokinetic parameters for lamotrigine of reference product (R).

Parameter	N	Reference (R) (Mean ± SD)
C _{max} (ng/mL)	22	775.9925 ± 151.65352
AUC ₀₋₇₂ (ng.hr/mL)	22	24202.0993 ± 3742.95710
*T _{max} (hr)	22	0.875 (0.250 - 4.000)

*Expressed in terms of median (range)

Table 3: Pharmacokinetic parameters for lamotrigine test product (T).

Parameter	N	Test (T) (Mean ± SD)
C _{max} (ng/mL)	22	758.6062 ± 157.45250
AUC ₀₋₇₂ (ng.hr/mL)	22	24142.0312 ± 3641.69066
*T _{max} (hr)	22	1.165 (0.500 - 5.000)

*Expressed in terms of median (range)

DISCUSSION

The prime objective of the proposed bioequivalence studies is to ensure the safety and efficacy of generic formulations. Two formulations of the same drug are considered to be bioequivalent if they exhibit a comparison among the extent and rate of absorption, if they are administered in the same dose and similar experimental conditions.¹¹ In this study, the lamotrigine test and Lamotrigine reference products were evaluated to assess its bioequivalence. The results of the C_{max}, T_{max} and AUC of the reference is presented (Table 2) and of the test are

given (Table 3) their comparison showed that both products had (Table 4) no significant differences. The Linear plot of Geometric Mean plasmatic Lamotrigine concentration versus time points (N=22) curves from 0 to 70 hours is shown in figure 1 which shows no significant differences.

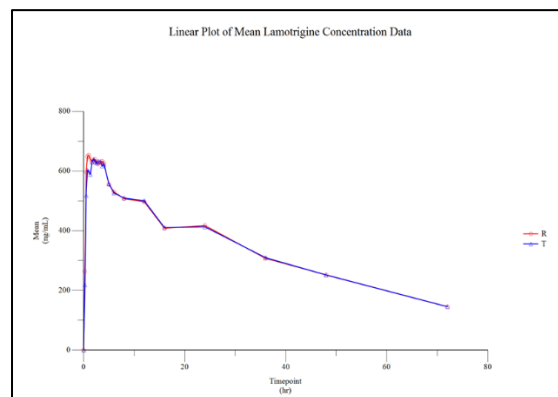


Figure 1: Linear plot of geometric mean plasmatic lamotrigine concentration versus time points (N=22) curves from 0 to 70 hours.

The sample size was calculated expected mean difference of 10% between the formulations, and the intra-subject CV of around 13.5%, 18 subjects would be required to prove bioequivalence at 80% power. On the basis of cross over design and considering possible dropouts due to expected adverse drug reaction (ADR), a sample size of 28 subjects was considered for pivotal bioequivalence study. However, due to insufficient of volunteer only 23 subjects were enrolled in the study. 18 subject's data can prove bioequivalence at 90% power. Hence, there is no impact on outcome of the study.

Table 4: Statistical results of test product (T) versus reference product-r for lamotrigine.

Parameters	Antilog Least	Square Mean	T/R Ratio (%)	90% Confidence Interval	Intra Subject CV	Power (%)
	Test Product (T)	Reference Product (R)				
Ln (C _{max})	744.404	762.5749	97.62	90.17% - 105.68%	15.36%	99.73
Ln (AUC ₀₋₇₂)	23872.3468	23915.3009	99.82	97.87% - 101.81%	3.79%	100.00

For Lamotrigine, analysis of variance (ANOVA) was performed on the Ln-transformed data of C_{max} and AUC₀₋₇₂ using PROC GLM of SAS® (version 9.4) software. The analysis of variance model included sequence, period and treatment (formulation) as fixed effect and subjects nested within the sequence as a random effect. The sequence effect was tested at the 0.10 level of significance. All other main effects were tested at the 0.05

level of significance against the residual error (mean square error/MSE) from the ANOVA as the error term using 90% confidence interval approach. Based on comparisons of the test and reference product for Ln-transformed C_{max} and AUC₀₋₇₂ data, the ratio of the least square mean was calculated, as well as the 90% confidence intervals for Ln-transformed C_{max} and AUC₀₋₇₂ were determined. Sequence effect was insignificant for C_{max} and AUC₀₋₇₂

with respect to p - value 0.0742 and 0.2719 respectively. Period effect was insignificant for Cmax and significant for AUC0-t with respect to p -value 0.5622 and 0.7965 respectively. Treatment (Formulation) effect was insignificant for Cmax and AUC0-72 with respect to p - value 0.6061 and 0.8767 respectively. Subject (Sequence effect) was significant for Cmax and AUC0-72 with respect to p – value 0.0468 and <0.0001 respectively. The statistically significant effects can be ignored considering the analysis approach adopted for two way, cross over study design. All other ANOVA effects were statistically insignificant (i.e. p> 0.05 and > 0.10) for Lamotrigine. These results were similar to Ruiz et al and that of Srichaiya et al.^{12,13}

CONCLUSION

Based on the results obtained, was concluded that Lamotrigine tablets of 25 mg of the test formulation and the reference formulation were bioequivalent.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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