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Research Article

A RANDOMIZED, CROSSOVER STUDY TO DETERMINE BIOEQUIVALENCE OF ESLICARBAZEPINE ACETATE IN HEALTHY INDIAN SUBJECTS

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ABSTRACT

To evaluate bioequivalence and pharmacokinetics of Eslicarbazepine between single dose of Eslicarbazepine tablets containing Eslicarbazepine acetate 800 mg (test formulation) and compare with single dose of ZEBINIX tablets containing Eslicarbazepine acetate 800 mg (reference formulation).

Subject and methods

In a two-way, two-period, two-sequence, randomized cross-over design, with ten days washout period between dosing, a single dose of test and reference formulations were orally administered in 26 healthy volunteers under fasting conditions. Twenty blood samples were drawn from each subject over 72 hours period. Analysis of subject sample was performed on High Performance Liquid Chromatography. C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were calculated by applying non-compartmental analysis. Data for test and reference formulations were analyzed statistically to test for bioequivalence.

Results

All 26 volunteers completed the study and provided adequate amount of blood at each sampling point. No adverse event was observed. The values of C_{max} (µg/mL), T_{max} (hr), $AUC_{(0-t)}$ (µg/mL*hr) and $AUC_{(0-\infty)}$(µg/mL*hr) for Eslicarbazepine in reference and test formulations were 15.3581 and 15.5269, 3.8654 and 4.6538, 337.9636 and 322.3675 and 350.3112 and 327.7690 respectively. Both clinical and laboratory parameters of all subjects showed no clinically significant changes.

Conclusion

Eslicarbazepine tablets containing Eslicarbazepine acetate 800 mg was bioequivalent to single dose of reference formulations and was well-tolerated.

Keywords: Eslicarbazepine acetate, Eslicarbazepine, Pharmacokinetics, Bioequivalence

INTRODUCTION

Eslicarbazepine acetate (ESL) {(S)-10-acetoxy-10,11-dihydro- 5H-dibenz[b,f]azepine-5- carboxamide}is a novel voltage-gated sodium channel (VGSC) blocker that is chemically related to carbamazepine and oxcarbazepine,[1-2] but with differences in metabolism that may result in lower drug interaction potential and a favorable safety profile.[3]

Following oral administration, ESL is rapidly metabolized to Eslicarbazepine (the entity responsible for the pharmacological effect)[4]

via extensive hydrolytic pre-systemic first pass metabolism.[5] The half-life of Eslicarbazepine in healthy subjects and epileptic adults is compatible with once-daily administration and consistent with an effective half-life of 20-24 h at steady-state plasma concentrations.[3] Mean Eslicarbazepine plasma ''trough'' (i.e., pre-dose) concentrations following once-daily administration of ESL 400 mg, 800 mg and 1200 mg in adult epileptic patients showed linearity and dose-proportionality.[6] ESL pharmacokinetics are unaffected by food, age or gender.[7-8] It has completed Phase III clinical trials as adjunctive therapy in partial epilepsy in adults. Other ongoing studies include Phase II in neuropathic pain, prevention of migraine and fibromyalgia.[9]

The present study aimed to provide information on the bioequivalence between the ESL 800mg tablet strengths of the test and reference formulation in healthy Indian subjects.

MATERIALS AND METHODS

The test product was a Eslicarbazepine tablet containing Eslicarbazepine acetate 800 mg manufactured by EMCURE PHARMACEUTICALS LTD., INDIA. The reference product was ZEBINIX tablet containing Eslicarbazepine acetate 800 mg manufactured by BIAL - PORTELA & Ca, SA.

The study was conducted as per ethical norms laid down in the Good Clinical Practice guidelines issued by Indian Council of Medical Research, New Delhi, 2000, and the Declaration of Helsinki, Seoul 2008, after obtaining approval from the Institutional Ethical Committee of the study center and signed written informed consent from the volunteers.

The study was conducted as a one center, open-labeled, randomized, two-way, two-period, two-treatment, single dose cross-over and comparative design with 10 days was-out period between dosing. Volunteers for this study were selected after successful completion of medical examination including medical history, complete physical examination, electrocardiogram and a laboratory profile with hematological, urine and biochemical tests. Volunteers were excluded if they were possibly sensitive to any of the study medications or related products, had significant medical disorder (cardiovascular, gastrointestinal, renal, hepatic, pulmonary, hematological, neurological, and psychiatric); had a history or evidence of drug abuse; had a history of alcohol consumption or smoking; had a positive screening test for any one or more: HIV, hepatitis B, VDRL, hepatitis C; had a history of consumption of xanthine-containing products, tobacco containing products or alcohol within 48 hours prior to dosing; had a history of requirement of any medication for chronic illness; had a history of consumption of any medication (prescribed or over-the-counter) during 14 days prior to dosing; had a history of food allergy; had a history of participation in any clinical study during 90 days prior to administration of study medication; had a history of blood donation during 90 days prior to administration of study medication and had a history of clinically significant illness within 4 weeks before start of study.

Thirty non-smoking, healthy, adult, male volunteers of Indian origin, with mean age of 32.4231 ± 1.4263 years and weight of 64.0000 ± 1.5252 kg, completed the study. Subjects were confined at the

clinical facility at least 13 hours before and till 24 hours after dose administration (for each study period). They visited the clinical facility for the ambulatory samples as required. A standard dinner was served to the subjects at least 10 hours before dosing. An indwelling venous cannula was introduced in the subjects left/right forearm vein and pre-dose blood sample was collected in pre-labeled centrifuge tubes with K_2-EDTA an anticoagulant. The drug was administered with 240 ml of water after an overnight fasting.

Post-dose sampling time points after formulation administration were 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 6.00, 8.00, 10.00, 12.00, 14.0, 18.00, 24.00, 48.00 and 72.00 hour. The actual collection time of each blood sample was noted accurately to nearest minute. The cannula was kept patent by injecting 0.5 ml of normal saline solution after collection of each sample. Blood samples were collected after discarding the first 0.5 ml of blood from the cannula from 0.5 hour till 24 hour post-dose on each occasion. Remaining samples were withdrawn by direct vein-puncture. Blood samples were centrifuged to separate plasma within half hour of collection at 4000 rpm for 10 minutes at around 0-4^{o}C. Plasma was separated in duplicate and placed in coded polypropylene vials, stored at -20± 5^{o}C. For control of the storage temperature the freezer temperature was checked thrice daily (in the morning, afternoon and in the evening) as per the standard operating procedure.

Standardized meals were provided to the subjects at the end of 4, 8 and 14 hours after dosing. Respective meal contents were identical in both periods. No water was permitted 1 hour before and 2 hours after dosing. Subjects abstained from alcohol for each study run and no consumption of alcohol was permitted from 48 h prior to dose administration till the end of follow-up examination. Body temperature, blood pressure, pulse rate and respiratory rate were measured at regular intervals. Emergence of symptoms, if any, was noted, by asking the subjects during and at the end of the study.

RP-HPLC assay of Eslicarbazepine in plasma

Eslicarbazepine concentrations were measured using validated RP-HPLC method in accordance with the principle of good laboratory practices. The liquid-liquid extraction method was used. The linearity range for eslicarbazepine was 0.10 to 30.00 µg/mL. The validation parameters included sensitivity, specificity, ruggedness, calibration range curve, precision, accuracy, recovery and stability. Stability of eslicarbazepine in plasma was evaluated as freeze-thaw cycle stability, bench top (room temperature) stability, short term stability and long term stability. In addition, short and long term stock solution stability and auto-sampler stability were also evaluated. The acceptance criteria for the validation was followed as per standards of the FDA validation guidlines.[10-11] The developed liquid-liquid extraction procedure was found to be simple, robust and provide high recovery rate, resulting in a fast and easily-handled analysis. All concentration values below the limit of quantification (BLQ) were reported as zero for all pharmacokinetic and statistical evaluation.

Pharmacokinetic and statistical analysis

Eslicarbazepine plasma levels data and pharmacokinetic output from SAS software (Version 9.1-Revision 9.1.3 SAS Institute, USA) and Microsoft Office 2000, were analyzed statistically. The statistical analysis was performed using SAS software (Version 9.1-Revision 9.1.3 SAS Institute, USA) and Microsoft Office 2000. The following statistical analyses were performed: descriptive statistics C_{max}, $T_{max,}$ $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were reported for both the investigational products. Analysis of Variance (ANOVA),[12] two-one sided 't' tests for bioequivalence for log transformed pharmacokinetic parameters (C_{max}, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$) and Ratio analysis of untransformed and log transformed pharmacokinetic parameters of (C_{max}, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$) were carried out. In addition the terminal phase elimination rate constant (K_{el}) and elimination half-life ($T_{1/2}$) were also determined. For bioequivalence evaluation, in accordance with current FDA guidelines, the products were considered bioequivalent if the 90% confidence interval for C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ fell within the range of 80% to 125%.[13-15]

RESULTS AND DISCUSSION

Twenty six healthy male Indian volunteers were enrolled in the study and all volunteers completed the study. Both the formulations of eslicarbazepine acetate was well tolerated by the volunteers in both periods of the study with no adverse effects reported or observed. All volunteers continued to the end and were discharged in good health.

Table 1: Back calculated concentration of calibration sample for eslicarbazepine

Calibrant Samples For Eslicarbazepine (ESL III)							
Curve code	**0.10**	**0.50**	**1.00**	**4.00**	**8.00**	**20.00**	**30.00**
	µg/ml	**µg/ml**	**µg/ml**	**µg/ml**	**µg/ml**	**µg/ml**	**µg/ml**
Day 1	0.10	0.50	1.03	3.78	8.22	19.65	30.32
Day 2	0.09	0.51	1.06	3.99	8.18	19.60	30.17
Day 3	0.10	0.48	1.09	3.97	8.18	19.19	30.60
Mean	0.096	0.497	1.060	3.913	8.193	19.481	30.361
S.D.	0.0039	0.0136	0.0286	0.1187	0.0209	0.2565	0.2173
% C.V.	4.11	2.73	2.70	3.03	0.26	1.32	0.72
% Nominal	95.81	99.35	106.01	97.82	102.41	97.40	101.20
Criteria For % Nominal	For CC standards other than LLOQ 85% - 115 %				For LLOQ 80% - 120 %		

Table 2: Descriptive statistics of the pharmacokinetic parameters of Single dose of Eslicarbazepine tablets, containing Eslicarbazepine acetate 800 mg (Test Formulations) and Single dose of ZEBINIX tablets containing Eslicarbazepine acetate 800 mg (Reference Formulations) administered to 26 healthy adult male subjects.

Pharmacokinetic Parameters	**Reference**				**Test**			
	Mean	**S.D.**	**S.E.**	**%CV**	**Mean**	**S.D.**	**S.E.**	**%CV**
C_{max} (µg/ml)	15.3581	3.1733	0.6223	20.6620	15.5269	3.8333	0.7518	24.6879
$AUC_{(0-t)}$ (µg/ml*hr.)	337.9636	85.6210	16.7917	25.3344	322.3675	61.9337	12.1462	19.2121
$AUC_{(0-\infty)}$ (µg/ml*hr.)	350.3112	97.8293	19.1859	27.9264	327.7690	63.3112	12.4164	19.3158
C_{max} / $AUC_{(0-\infty)}$ (hr^{-1})	0.0453	0.0084	0.0016	18.5623	0.0476	0.0081	0.0016	16.9520
T_{max} (hr)	3.8654	1.7062	0.3346	44.1408	4.6538	1.7650	0.3462	37.9266
K_{el} (hr^{-1})	0.0596	0.0145	0.0028	24.3145	0.0623	0.0161	0.0032	25.8807
$T_{½}$ (hr)	12.3647	3.3607	0.6591	27.1800	11.6958	2.4519	0.4809	20.9637
ln C_{max} (µg/ml)	2.7129	0.1941	0.0381	7.1563	2.7143	0.2416	0.0474	8.9008
ln $AUC_{(0-t)}$ (µg/ml*hr.)	5.7944	0.2399	0.0470	4.1401	5.7578	0.1942	0.0381	3.3733
ln $AUC_{(0-\infty)}$ (µg/ml*hr.)	5.8253	0.2584	0.0507	4.4352	5.7743	0.1948	0.0382	3.3728
ln (C_{max} / $AUC_{(0-\infty)}$)(hr^{-1})	-3.1124	0.1956	0.0384	-6.2851	-3.0600	0.1783	0.0350	-5.8277

The analytical method for eslicarbazepine plasma sample showed good specificity, sensitivity, linearity, precision and accuracy. The linearity was observed within the range of 0.10 to 30.00µg/mL (Table 1) with the coefficient of correlation varied from 0.9987 to 0.9997. The between and within batch precision for all the low, middle and high quality control samples of eslicarbazepine were ranged from 90.67% to 104.97% and 90.47% to 105.96% respectively, which are within the acceptance limit of 85% to 115%. Stock solution stability observed at room temperature was 12hrs and 15days long term stability at 2-8 °C. Plasma samples were stable for 15 days at -20 ± 5°C. Consistent recoveries are observed for lower and higher quality controls samples. All the stabilities performed were under acceptance criteria as per standard guidelines of ANVISA and FDA.[10-11] The method is specific enough in the presence of different matrices collected from different sources. This method can be used for quantification of eslicarbazepine in human plasma for bioequivalence studies. The plasma levels produced by the administration of the test and reference formulations in each subject were used to establish the pharmacokinetic profile of all formulations (Figure 1). The calculated pharmacokinetic parameters of eslicarbazepine are shown in Table 2. C_{max}, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and $C_{max}/AUC_{(0-\infty)}$ values were comparable in both formulations. The T/R ratio of geometric mean of C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ for eslicarbazepine in both formulations were 100.15%, 96.41% and 95.03% respectively (Table 3).

Table 3: Geometric mean for Eslicarbazepine acetate 800 mg table (test and reference)

Pharmacokinetic parameters (Eslicarbazepine)	Geometric Mean		% Ratio of (Test / References)
	Reference	Test	
C_{max} (µg/ml)	15.0720	15.0950	100.15
$AUC_{(0-t)}$ (µg/ml*hr.)	328.4520	316.6540	96.41
$AUC_{(0-\infty)}$ (µg/ml*hr.)	338.7570	321.9210	95.03

Table 4: 90% confidence interval for the pharmacokinetic parameters of Eslicarbazepine acetate 800 mg tablet (test versus reference)

Data	90% Confidence Interval		Accepted 90% Confidence Interval	
	Lower	Upper	Lower	Upper
ln C_{max}	93.59	107.17	80.00	125.00
ln $AUC_{(0-t)}$	89.59	103.74	80.00	125.00
ln $AUC_{(0-\infty)}$	87.83	102.83	80.00	125.00

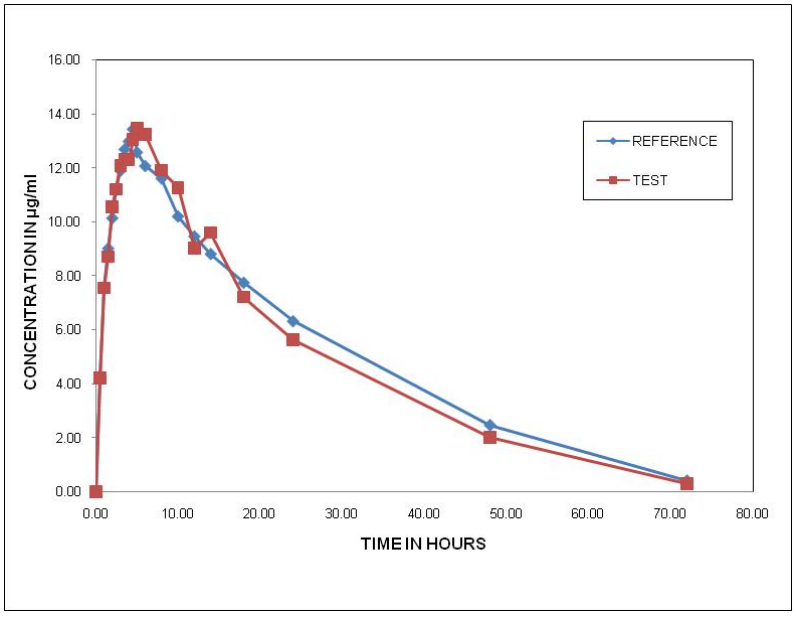

Fig. 1: Plasma concentration of Eslicarbazepine acetate 800 mg tablet (test and reference)

These values were within the acceptance limit of 80-120%. ANOVA assessment found no significant product, group or period effect in the present study. The 90% confidence interval for the ratio of C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ values for the test and reference products fell within the established regulatory interval of 80%-125% (Table 4).

CONCLUSION

In this study, both formulations were well tolerated. No statistically significant differences in C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were found between the Eslicarbazepine tablets, containing Eslicarbazepine acetate 800 mg (manufactured by, EMCURE PHARMACEUTICALS LTD., INDIA) and ZEBINIX tablets containing Eslicarbazepine acetate 800 mg (manufactured by BIAL - PORTELA & Ca, SA). The reported data were entirely within the bioequivalence acceptance range proposed by the FDA of 80%-125%. The test formulation can be considered a pharmaceutically and therapeutically equivalent alternative.

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