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Research Article

A Two-Sequence, Four-Period, Crossover, Full-Replicate Study to Demonstrate Bioequivalence of Carbamazepine Extended-Release Tablets in Healthy Subjects under Fasting and Fed Conditions

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Abstract

Carbamazepine is a first-line antiepileptic drug (AED) used for the treatment of partial and tonic-clonic seizures. We conducted an open label, balanced, randomized, two-treatment, two-sequence, four-period, single oral dose, full-replicate crossover study to assess and compare the bioequivalence of test product Carbamazepine extended release tablets USP 400 mg with reference product Tegretol®-XR 400 mg (Carbamazepine extended release tablets), respectively in healthy subjects under fasting and fed conditions. Blood samples were collected pre-dose and at regular intervals post-dose up to 240.00 hours. The plasma concentration was analyzed by a validated LC-MS/MS method and the reference-scaled and the unscaled procedure was used to determine bioequivalence for the pharmacokinetics parameters, C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, $T_{½}$, Ke_l and AUC extrapolated was calculated. The results showed that the geometric mean ratios of C_{max}, AUC_{0-t} and AUC_{0-inf} were 113.04%, 108.33% and 108.15% respectively, in the fasting conditions and 113.99%, 110.13% and 111.41%, respectively, in the fed conditions and the 90% confidence intervals were all within the range of 80.00% to 125.00%. It can be concluded from the result that the test product Carbamazepine extended release tablets based on osmotic release system (OROS) are bioequivalent to the reference product Tegretol®-XR tablets.

Keywords: Bioequivalence, Carbamazepine, Sodium Channel Modulators and Epilepsy

INTRODUCTION

Epilepsy is a chronic neurological condition of the central nervous system that affects around 50 million people worldwide. Epilepsy accounts for a significant proportion of the world's disease burden and an estimate of 5 million people are diagnosed with epilepsy each year. This neurological disorder is characterized by the recurrence of unprovoked seizures due to excessive electrical discharge of neurotransmitters in a group of brain cells which lead to brief episodes of involuntary movement which may involve a part of the body (partial) or the entire body (generalized) [1, 2].

In the majority of cases, seizures can be due to underlying conditions such as neurological disorders, infectious diseases, or developmental disorders. In the past few years, the development of anti-epileptic treatment has provided a significant benefit by either eliminating seizures or reducing their frequency to the maximum degree possible. There are several anti-epileptic medications clinically used either as monotherapy or combined together[3].

Carbamazepine is an effective anti-seizure drug that is often used as a first-line agent in the treatment of epilepsy. Carbamazepine is also indicated for the management of other neurological disorders such as bipolar disorders, trigeminal neuralgia, attention-deficit hyperactivity disorder (ADHD) and schizophrenia albeit off-label.[4,5] Therapeutic anticonvulsant mechanism of Carbamazepine is primarily related to the blockade of presynaptic voltage-gated sodium channels and is believed to inhibit the release of synaptic glutamate and possibly other neurotransmitters. Carbamazepine is also a powerful inhibitor of the muscarinic and nicotinic acetylcholine receptors, N-methyl-Daspartate receptors, and the central nervous system adenosine receptors[6].

Carbamazepine absorption is slow and variable, but it is almost complete from the gastrointestinal tract. Time to peak concentration varies from 4 to 8 h or longer because of the very low water solubility (< 200 µg/ml) of this drug and its dissolution-rate-limited absorption. Carbamazepine has a half-life ($t_{1/2}$) which ranges from 25 to 65 h for an initial single dose.[7] The efficacious plasma concentration range for Carbamazepine is 4-12 µg ml^{-1}. Conventional release tablets

are commonly dosed 2 to 4 times daily up to total daily doses of 1600 to 2000 mg day $^{-1}$ to achieve the appropriate concentration. Concentrations outside the therapeutic range are generally associated with adverse effects or reduced efficacy.[8] Extended release dosage forms containing Carbamazepine, have been currently investigated with the aim to smooth out plasma fluctuations and extend drug action.[4] To facilitate patient adherence by reducing dosing frequency and minimizing plasma fluctuations, we at J.B. Chemicals & Pharmaceuticals Ltd have developed an osmotic release system (OROS) formulation of Carbamazepine extended-release tablets USP 400 mg which is pharmacokinetically equivalent to commercially available Tegretol®-XR by Novartis Pharma GmbH Wehr, Germany.

Thus, this study assesses the pharmacokinetics of the test product Carbamazepine extended release tablets USP 400 mg and the branded reference product Tegretol®-XR 400 mg after administration of a single dose under fasting and fed conditions in healthy subjects. As Carbamazepine is considered a narrow therapeutic index drug, we designed a two-sequence and four-period crossover, replicate study to get the reliable intra subject variability for the test and reference products. the bioequivalence acceptance limit could be adjusted based on the reference-scaled average bioequivalence method, according to US Food and Drug Administration draft guidance on the investigation of bioequivalence for narrow therapeutic index drugs.[9]

MATERIAL AND METHODS

Design

An open label, balanced, randomized, two-treatment, two-sequence, four-period, single oral dose, full-replicate, crossover bioequivalence study of the test product (Carbamazepine), and the reference product (Tegretol®-XR), in healthy, adult human subjects under fasting and fed condition.

Objective

Pharmacokinetics: To compare the single dose oral bioequivalence of the test product, Carbamazepine extended-release tablets USP 400 mg manufactured by Unique Pharmaceutical Laboratories (A Division of J. B. Chemicals & Pharmaceuticals Ltd.), India and Reference product of Tegretol®-XR 400 mg (Carbamazepine extended-release tablets) manufactured by Novartis Pharma GmbH Wehr, Germany in healthy, adult, human subjects under fasting and fed condition.

Safety: To assess the safety and tolerability profile of test and reference products.

Number of Subjects

Fifty-two and 64 subjects were randomly assigned to sequence A (ABAB) and sequence B (BABA) and received drug under fasting and fed conditions. The volunteers aged from 18 to 45 years, had a weight not less than 50 kg and a body mass index in the range of 18.50 – 29.99 kg/m^2. Compliance with pre-defined inclusion and exclusion criteria was checked before the study started. The inclusion of subjects was based on demographic data, medical and surgical history, physical examination, 12-lead ECG, vital signs, and clinical laboratory investigations. Information about the study was given in writing and orally, and all subjects participating in this trial signed the informed consent. The exclusion criteria included history or presence of significant gastric and/or duodenal ulceration. Those who had participated in other clinical trial within 3 months prior to this study or who had blood loss of >500 mL in the past 8 weeks before this study were excluded. Subjects must not use any medication, vitamins and/or herbal supplements, within 30 days prior to study drug administration.

Ethical Considerations and Informed Consent

The present study was conducted in compliance and in accordance with the ethical principles that have their origins in the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research involving Human Subjects, revised by the 64th World Medical Association – General Assembly, Fortaleza, Brazil, October 2013), Good Clinical Practice (International Council on Harmonization – E6 (R1) Guidelines, Step 4 – 1996), along with "USFDA Guidelines", Schedule-Y (Amended Version, 2005 along with the 2013 amendments, Central Drugs Standards Control Organization, India), and Ethical Guidelines for Biomedical Research on Human Participants, 2006 – Indian Council of Medical Research (ICMR). The study did not commence until the IEC approved the protocol with corresponding ICFs. No subjects were enrolled in the study without obtaining written informed consent and were under medical supervision throughout their stay in the clinical facility to ensure their safety and well-being.

Safety Analysis

Safety monitoring including adverse events, serious adverse events, intercurrent illness (corresponding concomitant drugs) and laboratory assessments were performed during the entire course of the study. Adverse events were evaluated based on frequency, severity grades, system specific and causality.

STUDY PROCEDURE

Treatment Administered

A single oral dose of test product of Carbamazepine extended-release tablets USP 400 mg or reference product of Tegretol®-XR 400 mg (Carbamazepine extended-release tablets) was administered at 0.00 hours of each period with 240 ml of water at ambient temperature in sitting upright position as per randomization schedule. Subjects received the alternate 'treatment' in each period. Water was provided as desired except for 1.00 hour before and after drug administration, and subjects were instructed to remain in the supine position for at least 4.00 hours post-dose. thereafter, the subjects were allowed to engage in normal activities while avoiding strenuous physical activity, in such a way that each subject received both the 'treatments' by the end of the study. There was a washout period of 21 days between consecutive dosing and the housing of volunteers was 10.00 hours before dosing and until 48.00 hours post-dose.

Blood Sampling and Storage

Serial blood samples were collected from each subject via an indwelling cannula placed into the forearm antecubital vein for 24 hours post-dosing to avoid multiple skin punctures. Prior to each sample collection, 0.3 mL of blood was discarded, and cannula patency was maintained by flushing 1.00 mL of isotonic saline after each sample collection. The pre-dose blood sample were collected within 2 hours before dosing. Blood samples of 5 mL were collected in pre-labeled vacutainers containing K_3EDTA as an anticoagulant during each period. For fasting, the blood samples were collected during the study at sampling hours at pre-dose (0.00) and then at 1.00, 2.00, 3.00, 4.00, 6.00, 9.00, 12.00, 15.00, 18.00, 20.00, 22.00, 24.00, 26.00, 28.00, 30.00, 48.00, 72.00, 96.00, 120.00, 144.00, 168.00, 192.00 and 240.00 hours post dosing. A total of 24 blood samples were obtained from each subject in each period and for fed, the blood samples were withdrawn at pre-dose (0.00) and then at 1.00, 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00, 22.00,

24.00, 30.00, 36.00, 48.00, 96.00, 192.00 and 240.00 hours post dosing. A total of 23 blood samples were obtained from each subject in each period. In-house and ambulatory blood samples were collected within ±2 minutes and ±2.00 hours of scheduled sampling time, respectively. Blood sample withdrawn after >2 minutes for in-house samples or >2.00 hours for ambulatory samples from scheduled sampling time were reported as protocol deviations. After collection, blood sample vacutainers were centrifuged at 4000 RPM for 10 minutes at 2-8 °C to separate plasma. After centrifugation, plasma samples were separated into pre-labeled polypropylene tubes, in duplicates (Analytical and Replicate) as early as possible and were stored in a deep freezer maintained at -70°C±10°C immediately. until analysis. Carbamazepine is a light - sensitive drug, hence all activities where the drug was likely to be exposed to light such as pharmacy activities dispensing, dosing, blood sample collection, sample handling, processing and bioanalysis were performed under sodium vapor lamp(s).

Safety Assessment

During the entire study, subjects were continuously monitored for adverse events (AEs). Vital signs measurements (blood pressure, pulse rate, axillary and respiratory rate) and general well-being were assessed within 2.00 hours prior to dosing and, at 2.00, 6.00 12.00, 24.00 and 36.00 hours post-dose (±45 minutes of scheduled time) during the in-house stay of subjects. Physical examination, vital signs measurements and wellbeing were evaluated at check-out during each study period. Vital signs measurements and wellbeing were evaluated during the occurrence of any AE or termination/ withdrawal of subject from the study.

Analytical Method

Plasma samples were analyzed to quantify the concentration of Carbamazepine using a LC-MS/MS method.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters were subjected to a non-compartmental model using WinNonlin® - Software, Professional Version 5.3 or higher (Pharsight Corporation, USA) for PK analysis: Primary PK parameters: C_{max}, AUC_{0-t} and AUC_{0-inf} and secondary PK parameters: T_{max}, $t_{1/2}$, K_{el} and AUC extrapolated and statistical analysis was performed on PK parameters using Statistical Analysis Software - SAS® Version 9.4 or higher. Consistent with Schuirmann's two one-sided tests procedure for bioequivalence using reference-scaled & unscaled (classical 90% CI) average bioequivalence approach, ANOVA was performed on natural log-transformed PK parameters C_{max}, AUC_{0-t} and AUC_{0-inf} for Carbamazepine.

Criteria for Evaluation

A mixed-scaling average bioequivalence approach was used for the natural log-transformed PK parameters C_{max}, AUC_{0-t} and AUC_{0-inf} of narrow therapeutic index drug Carbamazepine. The assessment of bioequivalence was based on both reference-scaled average bioequivalence approach and also unscaled average bioequivalence approach bioequivalence limits i.e. the 90% confidence limits should be at least 80.00% and not more than 125.00%.

RESULT

Table 1: The mean and standard deviation (SD) of pharmacokinetic parameters estimated for average test product and average reference product

Pharmacokinetic parameters (units)	Mean ± SD (CV %)			
	Test product (A) (N = 46)	Reference product (B) (N = 45)	Test product (A) (N = 51)	Reference product (B) (N = 50)
	Fasting		Fed	
C_{max} (ng/mL)	3862.50±1193.91 (30.91)	3271.82 ± 959.38 (29.32)	5203.93 ± 964.66 (18.54)	4562.50±1067.01 (23.39)
AUC_{0-t} (ng.hr/mL)	324397.27±91971.95 (28.35)	291942.11±98606.82 (33.78)	472034.85±103679.34 (21.96)	425180.13±103685.14 (24.39)
AUC_{0-inf} (ng.hr/mL)	347392.26±102163.26 (29.41)	319278.97±122180.92 (38.27)	511718.22±123846.49 (24.20)	457095.08±116618.73 (25.51)
$t_{1/2}$ (hr)	57.88±11.36 (19.62)	58.75±12.46 (21.21)	19.00 (9.00-36.00)	18.25 (6.50 - 42.00)
K_{el} (hr^{-1})	0.0125±0.0026 (21.07)	0.0124±0.0027 (21.82)	0.0110±0.0029 (25.9740)	0.0116±0.0033 (28.1371)
T_{max} (hr)*	18.51 (9.00 – 27.00)	19.00 (7.50 – 38.00)	59.55 ± 12.99 (21.80)	58.01 ± 12.00 (20.69)
AUC ratio (%)	92.80±5.08 (5.48)	92.72±4.70 (5.06)	93.18±3.92 (4.20)	93.33±3.74 (4.01)
AUC extrapolated (%)	7.20±5.08 (70.66)	7.28±4.70 (64.50)	6.82±3.92 (57.43)	6.67±3.74 (56.14)

Fasting Condition

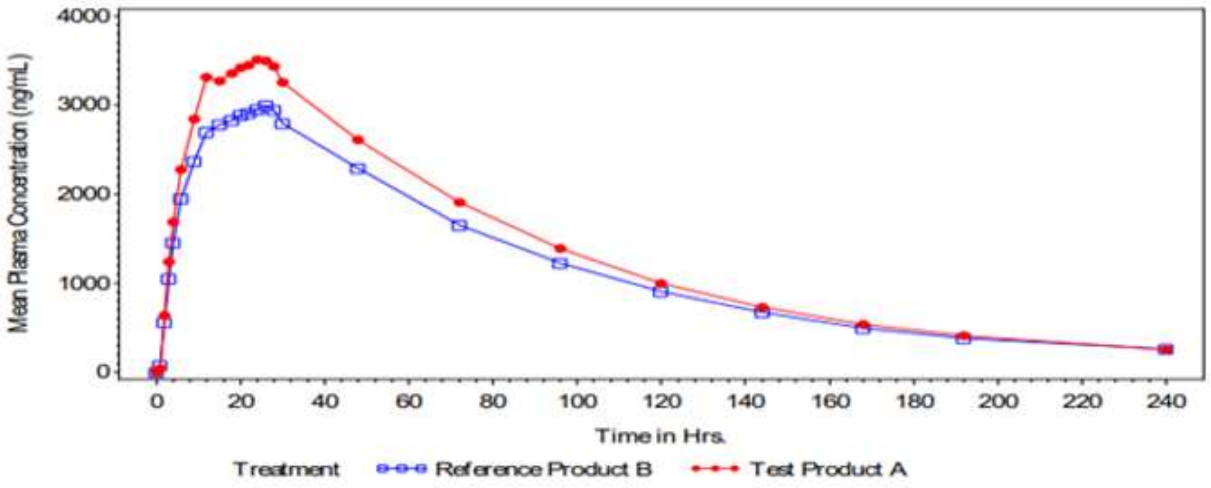

Figure 1: Mean plasma concentration (ng/mL) time profile of Carbamazepine for test product and reference product

Fed Condition

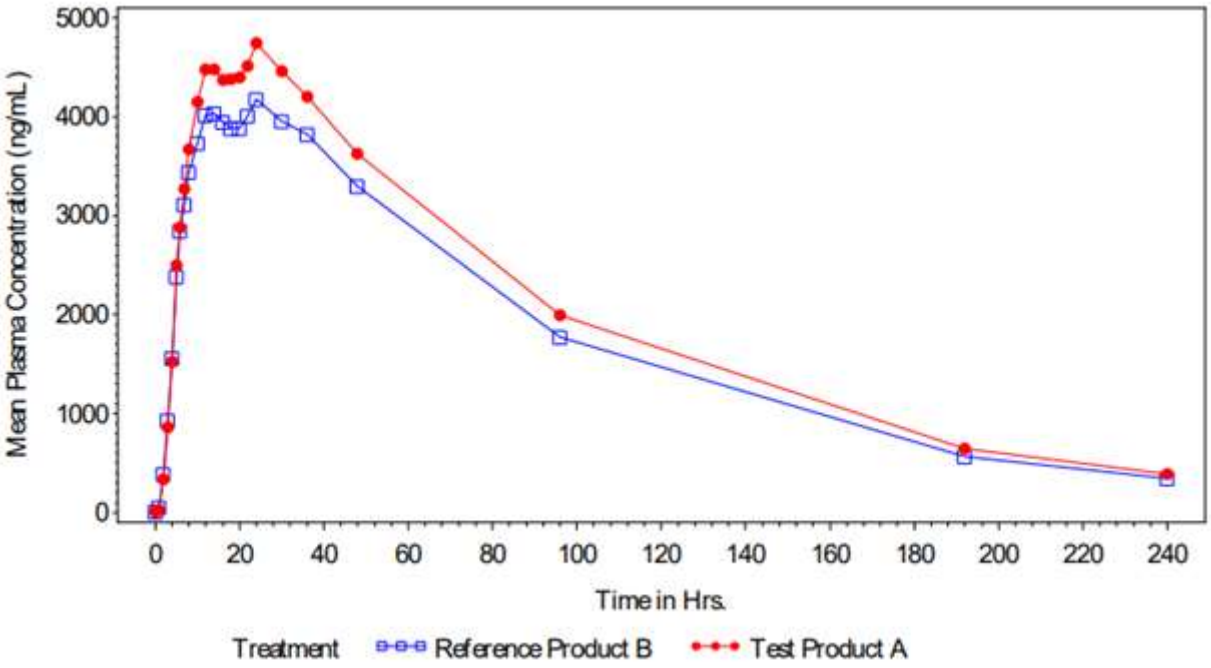

Figure 2: Mean plasma concentration (ng/mL) time profile of Carbamazepine for test product and reference product

DISCUSSION

Various pharmaceutical approaches have been made to design long acting dosage form to administer once a day formulation. In recent years, pharmaceutical research has led to the development of several novel drug delivery systems (NDDS). An osmotic drug delivery system is an NDDS designed to modulate and release a drug at a controlled rate over an extended period of time by utilizing osmotic pressure to drive drugs out that are suitable for oral administrations. The osmotic drug delivery system consist of a compressed tablet core that has a formulation of the drug which has a water-swellable polymer and an osmotic agent and is coated with a semi-permeable membrane coating. This semi-permeable coating has one or more than one delivery ports from which a solution of the drug or suspension of the drug is completely released over the duration of time. As the core absorbs aqueous content resulting to swell in terms of volume and compels the drug in form of solution or suspension through one or more than one delivery ports.[10]

The drug release of the osmotic system is typical zero-order release. In contrast with the conventional drug delivery system, osmotic pump tablets can maintain the stable plasma concentration and are independent of the presence or absence of food, pH of gastrointestinal. The key distinguishing feature of osmotic drug-delivery systems in comparison with other technologies used in controlled-release formulations is that they release the drug at a rate that is independent of the pH and hydrodynamics of the external dissolution medium. An additional critical benefit of the current osmotic systems is that they are related to drugs with a large variety of aqueous

solubility. Depending on aqueous solubility, the drug is released either as a solution or as a suspension. Any drug released as a suspension must dissolve in the *in vivo* environment and overcome biological barriers before it becomes systemically available.[11]

Osmotic release system can be used for both route of administration, that is, oral and implantation. The osmotic pump offers many advantages over other controlled drug delivery systems, that, they are easy to formulate and simple in operation, improved patient compliance with reduced dosing frequency and more consistency, and prolonged therapeutic effect with uniform blood concentration. Moreover, they are inexpensive and their production scale-up is easy.[12, 13]

We have compared the pharmacokinetic and safety profiles of Carbamazepine extended-release tablets, we designed a fully replicate study in which the subjects took both the test and reference products twice, overcoming the difficulty of the narrow therapeutics index of Carbamazepine. We could obtain reliable intra subject variability of both the test and reference products and use the reference scaled and unscaled average bioequivalence approach referencing FDA guidance on the investigation of bioequivalence. The assessment of bioequivalence was based on both reference-scaled average bioequivalence approach and unscaled average bioequivalence approach bioequivalence limits i.e., the 90% confidence limits should be at least 80.00% and not more than 125.00%. When calculating pharmacokinetic parameter in fasting condition, the within-subject standard deviation of reference for the pharmacokinetic parameters C_{max}, AUC_{0-t} and AUC_{0-inf} were 0.2794, 0.2873 and 0.3001 respectively and the 95% upper confidence bound of (μA -μB)2 – (θ* S2 WR) for the natural log-transformed was observed as -0.0196, -0.0408 and -0.0508 which was less than 0 and the upper limit of the 90% equal-tail confidence interval for σWT/σWR for C_{max}, AUC_{0-t} and AUC_{0-inf} was 2.2579, 2.4389 and 2.0588 respectively which was less than 2.5. In the fed condition, the within-subject standard deviation of reference for the pharmacokinetic parameters C_{max}, AUC_{0-t} and AUC_{0-inf} were 0.2006, 0.2389 and 0.2327 respectively and the 95% upper confidence bound of (μA -μB)2 – (θ* S2 WR) for the natural log-transformed was observed as -0.0039, -0.0306 and -0.0272 which was less than 0 and the upper limit of the 90% equal-tail confidence interval for σWT/σWR for C_{max}, AUC_{0-t} and AUC_{0-inf} was 1.1033, 0.9303 and 0.9189 respectively which was less than 2.5. Fifty-two and 64 healthy subjects were enrolled in this study under fasting and fed conditions. The drug administration and blood collection process were well followed. No drug-related serious adverse events occurred during the study in the fasting and fed conditions.

CONCLUSION

In this four-way-designed bioequivalence study, Carbamazepine extended-release tablets were shown to be safe and well tolerated in healthy subjects both under fasting and fed conditions. The current study demonstrates that the test product of Carbamazepine extended-release tablets based on the Osmotic release system (OROS) from JB Chemicals & Pharmaceuticals Ltd. (Mumbai) is bioequivalent to the reference product.

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CONFLICT OF INTEREST

BE study was a sponsored study from J B Chemicals & Pharmaceuticals Ltd.

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