

Three-way, three-period, crossover bioequivalence study of single oral dose of three brands of 300 mg phenytoin sodium tablets marketed in India, on healthy Indian human volunteers

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ABSTRACT

Objective: To compare the bioavailability of two brands of phenytoin sodium tablets available in the Indian market using Eptoin™ as the reference. **Materials and Methods:** A randomized, assessor-blind, three-way crossover design study was carried out over a period of 6 months after approval from the Institutional Review Board (IRB). Twenty-two healthy male participants received a single oral 300 mg oral tablet of either of the formulations with a 2-week washout. Blood samples were collected predose and at regular intervals postdose. Plasma phenytoin levels were estimated by high-performance liquid chromatography. Calculation of C_{max} , AUC_{0-t} , and $AUC_{0-\infty}$ was done by the linear trapezoidal rule and 90-110% margin (90% confidence interval (CI)) was used to assess bioequivalence. **Results:** Twenty volunteers completed the study. It was seen that the log-transformed values of C_{max} , AUC_{0-t} , and $AUC_{0-\infty}$ of the test formulations were not within the specified limits. **Conclusion:** Bioinequivalence of available phenytoin brands indicates that switching brands could lead to variations in blood concentrations and thus impact safety and efficacy. If a brand switch is done for any reason, stringent drug-level monitoring is advised.

Key words: Bioequivalence, bioavailability, healthy volunteer, phenytoin sodium

INTRODUCTION

Epilepsy is a common chronic neurological disorder with a prevalence of 559/100,000 population and incidence of more than 100/100,000 population in the developing countries.^[1] Pharmacotherapy remains the mainstay of treatment and although

several new drugs have been introduced in the past decade, drugs like phenytoin continue to be widely prescribed in India.^[2] Being an old drug, as many as 26 generic versions of phenytoin are available in the market for physicians to choose from.^[3] Brand substitution for drugs like phenytoin, which follows nonlinear kinetics, could lead to loss of seizure control or adverse events due to alterations in bioavailability resulting from changes in excipients and formulation factor.^[2,4-7] Cost savings following switch to a generic product needs to be balanced against the possibility of changes in plasma concentrations, lower efficacy, adverse events, and poor adherence.^[5]

In spite of the large number of generic phenytoin brands available in India, only one study has compared the

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bioavailability of commonly prescribed formulations.^[2] Phenytoin continues to remain the first drug of choice for the treatment of epilepsy in India. In view of being an old drug, it does not undergo mandatory bioequivalence studies before marketing. It thus becomes important to compare the oral bioavailability of different marketed formulations periodically. Hence, the present study was carried out to compare bioavailability of a single 300 mg dose of two brands of phenytoin available in the Indian market using Eptoin™ (74% market share) as the reference.^[8]

MATERIALS AND METHODS

Ethics

The study was carried out after obtaining approval from the Institutional Review Board and registration at the Clinical Trial Registry of India (CTRI/2011/05/001709). Written informed consent was taken from all participants.

Study design

This was a randomized, double-blind (and assessor-blind) crossover bioequivalence study, with a three-treatment, three-period design. A Latin Square (computer generated [www.randomization.com]) randomization was used.

Investigational product

A single 300 mg dose of phenytoin sodium (three tablets of 100 mg each) was used. Eptoin was the reference (supplied by Abbott India Ltd.) and local brands B and C (generic formulations of phenytoin purchased from the retail market by the investigator) were tested in the present study. All formulations had more than 6 months of shelf life.

Study conduct

Twenty-two healthy male volunteers were enrolled after obtaining written and informed consent. Two volunteers withdrew consent during the course of the study and were excluded from the analysis. Inclusion criteria: Volunteers who were aged 18-45 years; body weight within 20% of the ideal body weight; normal physical examination (done within 2 weeks of the study), biochemical and hematologic test values (done within 1 week of the study), urine examination, and ECG – all within normal limits; HBsAg (Australia antigen) negativity; HIV negativity; and agreement to abstain from caffeine on the day of the study and from alcohol and any other medications for 48 h prior to entry into the study and during the course of the study. Exclusion criteria: History of chronic alcohol consumption or drug addiction or intercurrent or concurrent diseases, history of allergy or hypersensitivity to phenytoin sodium, consumption of tobacco in any form, participation in a new drug study in the past 6 months and in a bioavailability or any study of a marketed drug in the past 3 months, blood donation in the past 3 months, and any drug intake in the past 15 days or intake of an enzyme-inducing agent in the past 30 days.

Following inclusion, volunteers were admitted and fasted over night. Three 100 mg tablets (300 mg single dose) were administered together on an empty stomach by a pharmacist under the supervision of the investigator. Standardized meals were given 1, 4, and 12 h after drug intake. Blood samples were collected at the following time points: 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, 60, and 72 h and collected into heparinized tubes. Plasma was separated and stored at -70°C until pending analysis.

Pharmacokinetics and statistical analysis

PK Solution version 2.0 (Summit Research Centre, USA) were used to calculate pharmacokinetic parameters, which included C_{max} , t_{max} , AUC_{0-t} , and $\text{AUC}_{0-\infty}$. Area under the plasma concentration–time curve from time 0 to time t (AUC_{0-t}) was calculated by trapezoidal rule. Area under the plasma concentration – time curve from time 0 to infinity ($\text{AUC}_{0-\infty}$) where $\text{AUC} = \text{AUC}_{0-t} + C_t/k$, where C_t was the last measurable drug concentration at 72 h and k was the terminal elimination rate constant calculated using noncompartmental analysis.

Results of the three formulations were compared by analysis of variance (ANOVA) using Statistical Analysis Software (SAS) version (SAS Institute Inc., USA) 9.3 to observe effect of formulation, period, and sequence. Formulations were considered bioequivalent to reference formulation if their log (ln)-transformed values fell within 90% and 110% of the reference.

Chromatographic conditions

A specific reverse-phase high-performance liquid chromatography (HPLC) method for estimation of phenytoin without any interference from blank plasma was developed and validated for accuracy, precision, linearity, and stability as per US Food and Drug Administration (FDA) guidelines, May 2001. Dionex equipped with P680 HPLC pump, ASI-100 automated sample injector, UVD340U detector and Chromeleon software (Thermo Fisher India Pvt. Ltd.) and Inertsil column (C_{18} 150×4.6 mm, 5 μm) (GL Sciences, Inc. USA). Mobile phase consisted of water pH adjusted to 5.0 with 50 mM KH_2PO_4 (S.D. Fine, India): Methanol (Merck, India) (45:55) at flow rate of 1 ml/min with total run time for each sample 12 min. The samples were analyzed at detection wavelength of 220 nm using carbamazepine (Abbott India Ltd.) as internal standard. The lowest limit of quantification on the calibration curve that gave accuracy and precision within 20% of guideline limit was 0.5 $\mu\text{g/ml}$, while the lowest limit of detection was 0.1 $\mu\text{g/ml}$. The assay was found to be linear over the concentration range of 0.5-20 $\mu\text{g/ml}$. The samples were injected in duplicates. To ensure quality control (QC) of the assay, QC samples, Lower limit of quantification (low QC sample – 0.5 $\mu\text{g/ml}$), one near the center (middle QC sample – 8 $\mu\text{g/ml}$), and one near the upper boundary of the standard curve (high QC sample – 20 $\mu\text{g/ml}$) representing the entire range of the standard curve along with the calibration

curve were included in between each run. Along with the QC samples, each assay also included eight concentrations of the standard curve, blank sample, and one standard sample.

RESULTS

Demographics

The mean age of the participants was 25.9 ± 3.09 years. The mean weight, height, and body mass index (BMI) were 65.3 ± 7.07 kg, 170.3 ± 6.82 cm, and 22.54 ± 2.06 kg/m², respectively.

Safety and Tolerability: No adverse events were seen.

Pharmacokinetics

Table 1 summarizes C_{max} (µg/ml), t_{max} (h), AUC_{0-t} (µg h/ml), and AUC_{0-∞} (µg h/ml). Table 2 shows the 90% confidence intervals (CIs) of the ratios (test/reference) for the ln-transformed values of C_{max} (as an index of rate of absorption), AUC_{0-t}, and AUC_{0-∞} (as an index of the extent of absorption). The 90% CIs for the corresponding ratios of ln-transformed values of C_{max}, AUC_{0-t}, and AUC_{0-∞} were not within the 90-110% range. The lower and upper limits for 90% CIs for AUC₀₋₇₂ for drug B were 91 and 192; and for drug C, they were 103 and 205. Likewise, for AUC_{0-∞} they were 88 and 239 for drug B; and 96 and 201 for drug C. The limits of C_{max} for drug B were 92 and 175; and for drug C, they were 98 and 179. The phenytoin mean plasma concentration versus time curves of Eptoin™, drugs B and C are presented in Figures 1-4. Period effect and sequence effect for C_{max}, AUC_{0-t}, and AUC_{0-∞} and formulation effect for C_{max} and AUC_{0-∞} were not found significant (*P* > 0.05). Formulation effect for AUC_{0-t} was observed significant (*P* = 0.03).

Table 1: Pharmacokinetic parameters after single oral dose of 300 mg of phenytoin sodium				
Brand	C _{max} (µg/ml)	t _{max} (h)	AUC ₀₋₇₂ (µg h/ml)	AUC _{0-∞} (µg h/ml)
Eptoin	2.32±1.11	12.25±8.52	87.73±53.17	108.99±91.45
Brand B	3±1.83	13.95±11.11	120.63±107.51	316.14±801.38
Brand C	2.96±1.44	15.75±13.52	119.01±64.03	134.75±81.23

AUC=Area under curve

Table 2: 90% CIs of the ratios (test/reference) for the log-transformed values of pharmacokinetic parameters			
Parameter	Two one-sided 90% CI		
	Test/reference	Lower limit	Upper limit
AUC _{0-t} (µg h/ml)	B/Eptoin	0.91	1.92
	C/Eptoin	1.03	2.05
AUC _{0-∞} (µg h/ml)	B/Eptoin	0.88	2.39
	C/Eptoin	0.96	2.01
C _{max} (mg/ml)	B/Eptoin	0.92	1.75
	C/Eptoin	0.98	1.79

CI=Confidence intervals

DISCUSSION

The present study compared bioequivalence of a single 300 mg oral dose of two generic formulations of phenytoin (B and C) with Eptoin™ as reference in 20 healthy Indian male volunteers in a tertiary care center. It was observed that the ln-transformed values of C_{max}, AUC_{0-t}, and AUC_{0-∞} (90% CI) of B and C brands were not within the 90% and 110% limits of Eptoin™.^[9]

Several authors have studied bioavailability of phenytoin sodium in either volunteers or patients.^[2,10,11] In a study conducted on 17 patients in the United Kingdom, significant differences in

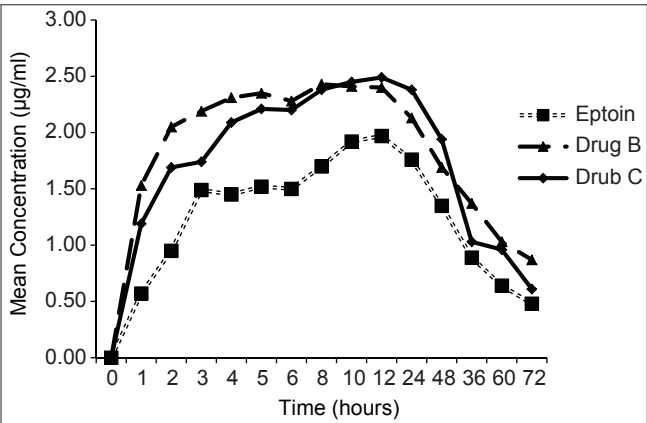


Figure 1: Phenytoin mean plasma concentration–time curve

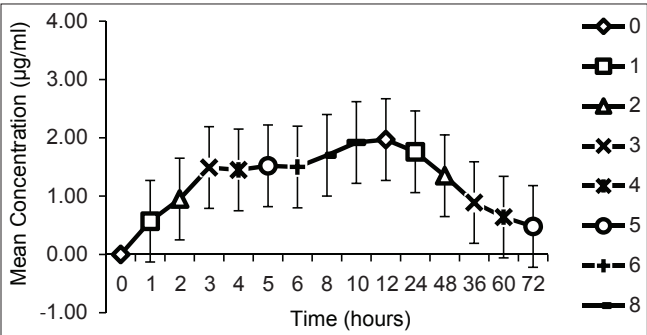


Figure 2: Mean plasma concentration–time curve of Eptoin™ showing standard deviation value

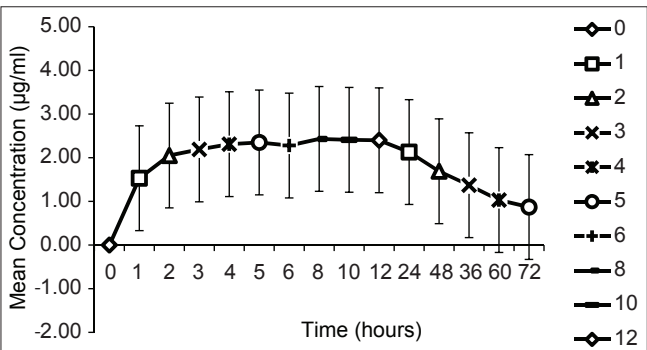


Figure 3: Mean plasma concentration–time curve of drug B showing standard deviation value

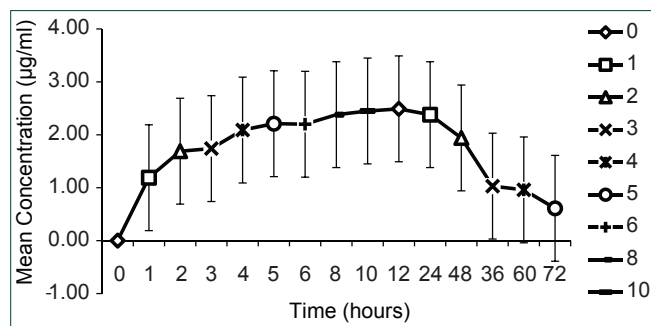


Figure 4: Mean plasma concentration–time curve of drug C showing standard deviation value

generic formulations were found, leading to the conclusion that switching of brands can lead to breakthrough seizures or adverse effects.^[11] Another group has compared bioavailability of three different brands of phenytoin with the innovator drug in 16 Thai healthy volunteers, and their results indicated that out of three brands, two brands were not bioequivalent to the innovator drug, and therefore, advised avoidance of brand interchanging.^[10] Several researchers have reported adverse impact of brand switching of various generic antiepileptic drugs and advocated avoidance of it.^[12-16] Brand switching is especially important in a country like India with the availability of at least 26 generics at varying costs (\$1.5-4/100 tablets).^[3,17] The less-expensive phenytoin generics, in spite of bioequivalence, may not always have therapeutic equivalence, and thus, breakthrough seizures or adverse events could result.^[4,18]

EptoinTM was selected as a reference product in this study as it is a widely prescribed antiepileptic medicine. The comparators B and C were also similarly chosen (14% and 2% market share, respectively).^[8] In the present study, the sample size of 20 was chosen as per Indian regulatory requirement and was also similar to other studies.^[2,10,11] It was observed, in this study, that brands B and C were not bioequivalent to the reference product EptoinTM. Several reasons could explain these variations, including the different excipients in the different formulations.

This study confirms the findings of a previous study performed on 12 healthy volunteers where the authors compared bioavailability of three brands of phenytoin (DilantinTM, EpsolinTM, and M-toinTM) to a reference product (EptoinTM).^[2] Overall, it was seen in both the studies that changing of brands can lead to alteration in the phenytoin plasma concentration.^[2,10-12] Hence, switching of brands should be avoided once the patient is stabilized with one medication. If switching is done, then it should be followed by therapeutic drug monitoring.^[2] One of the limitations of this study is the noninclusion of female participants, although we have found, in another study, that the number of females consenting to participate in nontherapeutic studies is limited.^[19]

CONCLUSION

Switching of brands in already stabilized patients can lead to alteration of plasma concentration of phenytoin in view of the fact that brands are not bioequivalent. In India, drug plasma concentration monitoring is not routinely carried out and thus switching should be avoided. Where unavoidable, it should be accompanied by therapeutic drug monitoring coupled with clinical observation.

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