Available online on 15.02.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Comparative bioavailability study of phenytoin in healthy Nepalese volunteers

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ABSTRACT

Our study aimed to assess and compare the bioavailability of Eptoin 100 mg and Epileptin 100mg tablets in Nepalese healthy volunteers. A randomized, two-treatment cross-over study with two weeks' wash-out period was conducted in 12 healthy non-smoker and non-alcoholic Nepalese male volunteers over a period of 6 months in the department of Clinical Pharmacology and Therapeutic at B. P. Koirala Institute of Health Sciences, Dharan, Nepal after approval from the Institutional Review Committee. The participants were randomized using sealed envelope system and received a single 100 mg oral tablet of either of the formulations with a two week washout period. Blood samples were collected predose and at regular intervals postdose upto 72 hours. Plasma phenytoin levels were estimated by reverse phase high performance liquid chromatography. The analytical method was validated prior to the start of study. C_{max} (Peak Plasma Concentration), T_{max} (Time to achieve maximum Plasma Concentration), AUC_{0-72} (Area under plasma concentration time curve 0 to 72 hours), $AUC_{0-\infty}$ (Area under plasma concentration time curve 0 to ∞) and $T_{½}$ (Elimination half-life) and K_{el} (Elimination rate constant) were calculated and 80-120% margin (90% confidence interval) was used to assess bioequivalence. ANOVA test was used to analyze the data at P-value of 0.05. All volunteers completed the study. The log-transformed values of C_{max}, T_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ of the both formulations were within the specified limits and were bioequivalent according to the regulatory definition of bioequivalence based on the rate and extent of absorption. Both products can be considered equally effective in medical practice.

Keywords: Bioavailability, Bioequivalence, healthy volunteer, Nepal, phenytoin sodium.

Article Info: Received 23 Dec 2018; Review Completed 02 Feb 2019; Accepted 03 Feb 2019; Available online 15 Feb 2019

Cite this article as:

Rai D, Rauniar GP, Comparative bioavailability study of phenytoin in healthy Nepalese volunteers, Journal of Drug Delivery and Therapeutics. 2019; 9(1-s):144-147 **DOI: http://dx.doi.org/10.22270/jddt.v9i1-s.2376**

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INTRODUCTION

Phenytoin is one of the most widely used drug in partial and tonic-clonic seizure. Its pharmacokinetic is changed from first-order to zero-order at high dose. Its rate of absorption also differs markedly with different brand formulation and hence its plasma concentration may vary and ultimately affect seizure control.[1,2] As various national and international brands of phenytoin are available in Nepal, their substitution may affect its bioavailability and affect the clinical response and cause intolerable adverse effects.[3,4] Some patient may switch to cheaper brand of phenytoin which may not be bioequivalent to the parent drug and it ultimately affect seizure control. The generic phenytoin was associated with an increase in serum concentration as compared to its branded formulation in a study conducted in the USA.[5] Changing from brand formulation of phenytoin to a generic has resulted in new seizure attacks. Physicians, pharmacists, patients and policy makers should be aware that for some patients there may be risks associated with switching from brand to generic formulation of phenytoin.[6]

It is the priority of the health care professionals and the policy makers to make the country self-reliant in essential drugs production and to ensure the availability of safe, effective, standard, and quality drugs at affordable price in quantities sufficient to cover the health needs of general population. To achieve the same objective, domestic pharmaceuticals should be promoted. In spite of the large number of phenytoin brands available in Nepal, none has compared the bioavailability of commonly prescribed formulations. The United States Food and Drug Administration (USFDA) considers two products to be therapeutic equivalents if they are bioequivalent.[7] Bioequivalent study is considered as the gold-standard method for comparing two brand formulation of same drug.[8] Single-dose bioequivalent studies are generally more sensitive than multiple dose studies.[9] Therefore this study was aimed to assess and compare the bioavailability of

Eptoin 100 mg (Acme formulation Pvt. Ltd., India) and Epileptin 100mg (Asian pharmaceuticals Pvt. Ltd., Nepal) in Nepalese healthy volunteers.

MATERIALS AND METHODS

Type of study and its Setting: A randomized, two-treatment cross-over study with two weeks' wash-out period was conducted in the department of Clinical Pharmacology and Therapeutic at B. P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal over a period of 6 months.

Selection of participants: This study was conducted in 12 healthy non-smoker and non-alcoholic Nepalese male volunteers (aged 17-45years and within 20% of their ideal body-weight). According to international guidelines bioequivalence study should be performed on a minimum of 12 subjects to ensure a power of at least 80%.[10] Exclusion criteria were history of hypersensitivity to phenytoin, history or presence of gastrointestinal, liver or kidney disease, or any other conditions known to interfere with the absorption, distribution, metabolism or excretion of common medications; history or presence of cardiovascular or hematological disease, any clinically significant illness during the 4 weeks prior to day 1 of this study, maintenance therapy with any drug or history of drug dependence, alcohol abuse, or serious neurological or psychological disease; participation in a new drug study in the last 6 months; HIV and Australian Antigen positive subjects; clinically relevant abnormal physical and/or clinical findings at the screening; any drug intake in the last 30 days; donated blood in the previous month.

Ethical approval: The ethical clearance was taken from the institutional ethical committee. This study was carried out in accordance with the clinical research guidelines established by the basic principles defined in the International Conference on Harmonization guidelines for Good Clinical Practice, Nepal Health Research Council guidelines for biomedical research on human subjects and the principles enunciated in the Declaration of Helsinki.

Study procedure: The study participants were randomized using sealed envelope system to avoid bias of treatment allocation. After overnight fasting of at least 10 hours, single dose of Eptoin 100mg tablet (reference drug) was given to 6 participants and Epileptin 100mg tablets (test drug) was given to the other 6 participants with 240ml water. A total of 10 blood samples were collected from anti-cubital vein at 0 hours (just before drug administration), 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 24.0, 48.0 and 72.0 hours in centrifuge tubes containing ethylene diamine tetraacetic acid. The blood samples were centrifuged immediately at 5000 r.p.m. for 10 minutes, the plasma was separated into duplicate polypropylene tubes and stored frozen at -20°C. Before assaying the plasma was allowed to reach room temperature, vortexed, centrifuged and the residual clot was removed.

Analysis by HPLC: The concentration of the drugs in the blood samples were analyzed by reverse phase high performance liquid chromatography (HPLC) method (Knauer HPLC, Germany). The analytical method was validated prior to beginning of the study. The column consisted of Knauer C18, 250 X 4.6mm, 5µ particle size with C18 guard column, stainless steel. The mobile phase used was acetonitrile. The flow rate used was 1 ml/minute. The samples were analyzed at detection wavelength of 215 nm. Fixed loop Rheodyne injector system fitted with a 20µl Rheodyne Loop was used. Integrating software was Clarity Chrome. Photodiode array detector (Smart line 2800) from Knauer, Germany was used. Calibration samples were prepared by spiking 480 µL of control human plasma with 20 µL of working stock solution of analyte. The plasma concentration-time profile of phenytoin was determined using the zero moment non-compartmental pharmacokinetics method. The plasma drug level profile was presented in graphical forms. The following pharmacokinetic parameters of test drug (Epileptin) and reference drug (Eptoin) were calculated for each subject: C_{max} (Peak Plasma Concentration), T_{max} (Time to achieve maximum Plasma Concentration), AUC_{0-72} (Area under plasma concentration time curve 0 to 72 hours), $AUC_{0-\infty}$ (Area under plasma concentration time curve 0 to ∞), $T_{½}$ (Elimination half-life) and K_{el} (Elimination rate constant).

Statistical analysis: Descriptive parameters mean and standard deviation (SD) were calculated using SPSS version 11.5. ANOVA test was applied on untransformed (C_{max}, AUC_{0-72}, $AUC_{0-\infty}$) and log-transformed pharmacokinetic data (C_{max}, AUC_{0-72}, $AUC_{0-\infty}$). P value of 0.05 or less was considered statistically significant. The products of phenytoin were considered to be bioequivalent if the 90% confidence interval of difference in the average values of logarithmic AUC and C_{max} between test and reference drugs was within the acceptable range of Log (0.8) to Log (1.25).[8,11,12]

RESULTS

The mean age, weight, height, and body mass index (BMI) of the participants were 33.67 ± 9.75 years, 62.58 ± 12.92 kg, 161.29 ± 4.20 cm and 24.09 ± 5.03 kg/m^2 respectively. There was no incidence of any adverse event during the study period. All the volunteers completed the study in good health. Pharmacokinetic parameters of Eptoin and Epileptin are given in Table 1.

Table 1: Pharmacokinetic parameters (mean) of Eptoin and Epileptin (n=12)

Pharmacokinetic parameters (mean±SD)	Brand formulation of Phenytoin		P-value
	Eptoin	Epileptin	
C_{max} (µg/ml)	1.882±0.725	1.920±0.696	0.235*
t_{max} (h)	6.750±5.723	5.917±1.832	0.126*
AUC_{0-72} (µg h/ml)	64.949±38.309	65.486±36.674	0.324*
$AUC_{0-\infty}$ (µg h/ml)	91.343±85.302	93.369±89.818	0.152*
K_{el} (h^{-1})	0.032±0.014	0.034±0.016	0.241*
$t_{1/2}$ (h)	28.139±17.935	28.496±23.968	0.148*

*Statistically not significant at P-value of 0.05

Time-concentration curve of Eptoin (test drug) and Epileptin (reference drug) is shown in figure 1.

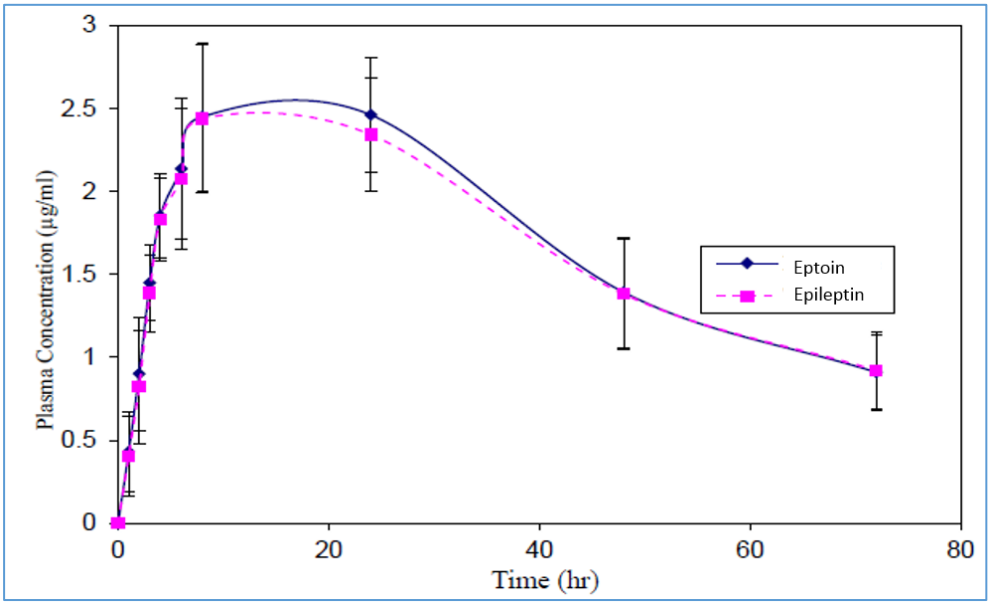

Figure 1: Mean time-concentration graph of twelve volunteers for test and reference preparation (n=12)

It was found that there was no statistically significant difference between test and reference drugs of in terms of C_{max}, lnC_{max}, T_{max}, lnT_{max}, AUC_{0-t}, $lnAUC_{0-t}$, AUC_{0-t}, ln $AUC_{0-\infty}$. Both drugs were found to be bioequivalent as 90% confidence interval for C_{max} and lnC_{max} (index of rate of absorption), AUC_{0-t}, $lnAUC_{0-t}$, $AUC_{0-\infty}$ and $lnAUC_{0-\infty}$ (index of extent of absorption) values of Epileptin (test drug) were within the accepted limit (80% to 125%) of that of the Eptoin (reference drug) as shown in Table 2.

Table 2: 90% Confidence Intervals of the ratios (Eptoin/Epileptin) of pharmacokinetic parameters (n=12)

Pharmacokinetic parameters	90% Confidence interval	
	Untransformed Data	Ln transformed Data
C_{max} (µg/ml)	0.93-1.11	0.88-1.23
AUC_{0-t} (µg h/ml)	0.96-1.06	0.99-1.02
$AUC_{0-\infty}$ (µg h/ml)	0.86-1.18	0.98-1.04

DISCUSSION

The US FDA has approved generic versions of phenytoin based on single-dose bioequivalence studies that required the 90% confidence intervals to fall within 80% to 125% of the originator product. The most important objective of bioequivalence study is to guarantee patients that generic products are safe and clinically effective within certain boundaries.[13-15] This study was conducted to compare the bioavailability of two tablet formulations of phenytoin sodium in twelve Nepalese healthy volunteers at a tertiary center in Eastern Nepal. It was a small scale randomized, two-way complete cross-over bioequivalence study with a two weeks wash-out period. After decoding the treatment allocation, twelve were in period I and twelve were in period II. All twelve subjects completed the study and received both the reference and the test drug alternately according to the randomization allocated in period I and period II. There was no dropout.

The maximum plasma concentration (C_{max}) of Eptoin 100mg tablet was 1.882±0.725 µg/ml at the time 6.750±5.723 hr (T_{max}) whereas the maximum plasma concentration of Epileptn 100mg tablet was 1.920±0.696 µg/ml (C_{max}) at the time 5.917±1.832 hr (T_{max}). The rate of absorption for the Eptoin (reference drug) and Epileptin (test drug) were similar as evidenced by their C_{max} and T_{max} values. The extent of absorption of the test and reference preparation were also similar as the plasma concentration time curve up to infinity ($AUC_{0-\infty}$) of reference drug was 91.343±85.302 µghr/ml, whereas that of the test drug was 93.369±89.818 µghr/ml.

Analysis of variance for log transformed pharmacokinetic parameters revealed that there was no significant effect of variation due to period and formulation for all the pharmacokinetic parameters. The study showed that both test and reference drug demonstrated comparable rate and extent of absorption. Overall, the test drug analyzed in the study satisfied the criteria for bioequivalence versus reference drug since the 90% CI interval of C_{max} and AUC_{0-t} and $AUC_{0-\infty}$ were within the specified limit of 80 to 120% for untransformed data and 80-125% for log-transformed data. Thus it can be claimed that test drug is bioequivalent to reference drug as per USFDA guidelines. The two formulations under the investigation demonstrated comparable rate and extent of absorption in healthy human volunteers under fasting conditions. Statistical analysis demonstrated that sequence and period effects did not occur. This finding partially supports another relevant issue on generic drug prescription as patients requiring long term drug treatment are likely to receive over time generic copies of the same active ingredient manufactured by different companies. With particular regard to drugs with narrow therapeutic index like phenytoin the results of the present study has to be cautiously interpreted in clinical setting with proper therapeutic drug monitoring as patients might be

subjected to variations of the steady-state pharmacokinetic parameters after multiple dosing.

Bioequivalence study of phenytoin has been done previously in different parts of the world by different researchers and institutions; however there were contradictory results on bioequivalence study. Gibberd et al studied the comparative bioavailability of two brands of phenytoin and found no significant difference between phenytoin levels for either preparation.[16] Similarly in another study, Meyer et al had determined inter-lot and intra-subject variability and effect of gender and menstrual cycle in the bioavailability of the 100mg extended phenytoin sodium capsules and found that there was very little difference in the bioavailability of the three lots of phenytoin.[17] In a study conducted in India, Gogtay et al had compared the bioavailability of a single oral 200mg dose of four brands (Dilantin, Epsolin, M-toin and Eptoin) of phenytoin and found that M-toin and Eptoin are bioequivalent but other brands were not.[18] These variations may be due to several factors including use of excipients in the different formulations. So, for inter-changeability of phenytoin only those preparations that are shown to be bioequivalent should be prescribed with therapeutic monitoring of the individual patients. Our study did not provide independent estimates of intra-subject variabilities since each subject received the same treatment only once. Female participants were not included as we did not get the written consent from them. As the data were obtained from healthy subjects who were administered a single dose, the pharmacokinetics parameters of phenytoin might differ in target populations. Effect on food on absorption was also not assessed.

CONCLUSIONS

Our study found that Epileptin 100mg was bioequivalent to Eptoin 100mg according to the regulatory definition of bioequivalence based on the rate and extent of absorption. Both products can be considered equally effective in medical practice and were well tolerated. Further studies are needed to compare these drug formulations in Nepalese patients. Therapeutic drug monitoring should be done during switching of brand formulation of phenytoin in epilepsy.

ACKNOWLEDGMENT

We would like to thank all the participants.

CONFLICT OF INTEREST

None declared.

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