Original article

Bioequivalence study of two different formulations of 300 mg gabapentin capsule in Thai healthy volunteers

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Abstract:

The objective of this study was to compare the bioavailability of new generic product of gabapentin with the innovator's product. The study was performed in 20 Thai male healthy volunteers who received a single oral dose of 300 mg gabapentin capsule. Double blind randomized two-way crossover design was used with one week washout period between treatments. After drug administration, serial blood sample was collected over a period of 32 hours. Plasma gabapentin was determined by automated high performance liquid chromatography (HPLC) with fluorescence detection after deproteinized with acetonitrile and following derivatization with o-phthalaldehyde (OPA) reagent containing 2-mercaptoethanol. The differences in pharmacokinetic parameters, maximum concentration (C_{max}), $AUC_{(0-t)}$ and $AUC_{(0-inf)}$ were analyzed by 2-way analysis of variance (ANOVA) and 90% confidence interval (CI). The values of C_{max} of gabapentin were 3.26 ± 0.60 (range of 1.58-4.07) μg/ml and 3.00 ± 0.71 (range of 1.42-3.95) μg/ml for generic and innovator's products, respectively. The time to peak plasma gabapentin concentration (T_{max}, hr) of generic and innovator's product was 3.08 ± 0.59 (2-4) hours and 3.33 ± 0.63 (2-5) hours, respectively. The area under the plasma concentration-time curve of generic and innovator's products were 30.06 ± 4.94 vs 27.63 ± 6.45 μg.hr/ml for $AUC_{(0-t)}$ and 30.76 ± 4.88 vs 28.27 ± 6.63 μg.hr/ml for $AUC_{(0-inf)}$, respectively. 90% CI of the difference of log C_{max}, log $AUC_{(0-t)}$ and log $AUC_{(0-inf)}$ of generic product compared to innovator's product were 96.64-124.94%, 97.60-124.43% and 97.90-124.00%, respectively. They were all within the acceptable range of 80-125%, thus we concluded that the two gabapentin formulations were bioequivalent.

Keywords: 300 mg Gabapentin; Bioequivalence; Capsule; Pharmacokinetics

Introduction

Gabapentin is an antiepileptic drug, which decreases neuronal excitability and may act by increasing the availability of gamma-aminobutyric acid. It has been used in many therapeutic areas such as antiepilepsy, neuropathic pain, psychiatric conditions both social phobia and panic disorder, antianxiety treatment of partial seizures in children.

Mean maximum plasma gabapentin concentrations are attained 2 to 3 hours after a single oral 300 mg dose, and measured 2.7-2.99 mg/l in healthy volunteers. Absorption kinetics of gabapentin are dose-dependent, rather than dose-proportional, possibly due to a saturable transport system. Thus, bioavailability of a single 300 mg oral dose of gabapentin is 60%, but decreases with increasing dose. As demonstrated in rats, gabapentin is extensively distributed in body tissues, concentrating particularly in pancreases and kidney. Unlike GABA, gabapentin has some lipophilicity and readily crosses the blood-brain barrier, producing a CSF: plasma concentration ratio of 0.09 to 0.14 as measured in 5 patients. Its volume of distribution is large, estimated as 50 to 60 l in healthy volunteers. The drug is not bound to human plasma proteins. Elimination of gabapentin is wholly accountable by renal clearance, in contrast to many antiepileptic drugs which are metabolised. The elimination half-life of gabapentin is about 5 to 7 hours after a single oral dose of 200 to 400 mg. As expected, renal impairment reduces drug clearance and augments plasma gabapentin concentrations in a linear fashion [1].

Mild adverse events, commonly somnolence, fatigue, ataxia and dizziness have been reported in about 75% of gabapentin recipients. Other events such as tremor, diplopia, nausea and vomiting were each experienced by < 10% of gabapentin recipients. The overall proportion of patients reporting adverse events during gabapentin administration has been calculated to be about 75%, versus 55% for placebo [1].

Currently, only the innovator's product of capsule containing gabapentin 300 mg is commercially available in Thailand. A new formulation of gabapentin 300 mg capsule is currently under development for local manufacture. The bioequivalence data of a new product with the innovator's product is required in order to assure its quality and performance.

Materials and Methods

Materials

Two drug products of gabapentin 300 mg capsule were used for in vivo bioequivalence study. One was the test product ("Test") locally manufactured lot no. K41GAB 01/1 and another was the "Reference" or innovator's product lot no. 0029084. Gabapentin, 1-(amino-methyl) cyclohexane acetic acid, was supplied by Siam Bheasach Laboratories Co., Ltd. Acetonitrile and methanol HPLC grade and monobasic potassium phosphate were obtained from MERCK. o-Phthalaldehyde (OPA) and 2-mercaptoethanol were obtained from Sigma Chemical Co., Ltd. Boric acid was purchased from BDH Chemicals Ltd. (Poole, UK).

Human subjects

The study was approved by the Ethic Committee of the Faculty of Medicine, Chulalongkorn University. Twenty healthy Thai male volunteers aged between 18-45 years were included in the study. All subjects were in good health confirming by physical and clinical laboratory examination including serology, hematology and biochemical test. All subjects were abstained from other drugs intake and alcoholic preparations two weeks prior to and throughout the study. Caffeine containing beverage was not allowed 3 days prior to and throughout the study. The subjects who had cigarrette smoking, alcoholic intake and caffeine intake habit were excluded.

Study design

The study was carried out according to a randomized, double blind, two-treatment, two-period, two-sequence, single dose crossover design with one week of drug-free interval between the periods. Each subject was prepared in the fasted state approximately eight hours prior to the study and randomly assigned to receive a single dose of 300 mg gabapentin with 200 ml of water.

On the study day a standardized lunch is consumed after the blood sampling at 4 hours. Blood samples were collected immediately before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 9, 12, 24 and 32 hours after drug intake. The plasma were separated by centrifugation and stored at -70 °C.

Analytical method

The analytical method was modified by the method of Tang *et al.* [2], Forrest *et al.* [3] and Chollet *et al.* [4] using automated HPLC with fluorescence detection. Chromatogra5phy was carried out at room temperature on Shimadzu-HPLC system-10AD series. A reversed phase column 250 x 4.6 mm i.d., C18, 5 µm ODS guarded with an inersil ODS-3, 5 µm was used. The mobile phase is consisted of 0.04M phosphate buffer (pH4.51)-acetonitrile (45:55, v/v) flowing through the system at the rate of 1.50 ml/min. The HPLC column temperature was 40 °C. Eluent was monitored by fluorescence with excitation and emission wavelengths of 230 and 420 nm, respectively. The sample injection volume was 20 µl.

300 µl of plasma was mixed with 1,200 µl of acetonitrile on a vortex mixer for 30 seconds and centrifuged at 1000 rpm for 5 minutes. 100 µl of supernatant, 100 µl of borate buffer pH 9.5 and 100 µl of derivatization reagents were mixing. 20 µl of derivatized sample was injected into HPLC system. Analytical method validation was modified from the method described by [5].

Data analysis

The pharmacokinetic parameter was determined. C_{max} and T_{max} were taken directly from the individual concentration versus time data. The elimination rate constant (K_{el}) was estimated by log-linear least squared regression of the terminal part of the plasma concentration versus time curve and half life ($T_{1/2}$) was calculated from the equation of 0.693/ K_{el}. The area under the concentration versus time curve (AUC_{0-inf}) was calculated by the linear trapezoidal rule.

The comparison of bioavailability of the generic product of 300 mg gabapentin to the innovators's product was assessed using the relevant pharmacokinetic parameters, C_{max}, AUC_{0-t} and AUC_{0-inf} which had been transformed to logarithmic scale before statistical analysis. The difference of the mean corresponding log C_{max}, log AUC_{0-t} and log AUC_{0-inf} between the two products will be determined by 2-way analysis of variance (ANOVA) for a crossover design at the significant level of $\alpha = 0.05$. The 90% confidence interval (CI) (two-one sided test) for the differences of the mean log C_{max}, log AUC_{0-t} and log AUC_{0-inf} between the two products were calculated.

The two products are considered to be bioequivalent when 90% CI of the differences of all parameters calculated above were within 80-125%.

Results

All subjects were judged to be healthy based on physical examination, medical history, vital signs and clinical laboratory test. The clinical laboratory data of all subjects enrolled in this are shown in Table 1. BMI of each subject was within the range of 18-25 kg/m^2.

The analytical method was validated to assure the acceptability of the performance. The parameters determined were lower limit of quantitation, accuracy, precision, selectivity, linearity and stability. Chromatogram of gabapentin is shown in Figure 1.

The lower limit of quantitation of the assay method was 0.025 µg/ml with 9.29 %CV (%CV<20%). The accuracy at low, medium and high concentrations were 98.34, 100.71 and 104.30%. The average %CV of intra-day and inter-day precision of gabapentin were 1.82% and 2.34%. Linear relationship between peak area ratio and drug concentration was observed (r^2>0.999) (concentration range from 0-4 µg/ml).

The means of plasma gabapentin concentration at each sampling time up to 32 hours following a single oral dose of 300 mg Test and Reference were presented by the graphic profile curve of mean ± SEM of plasma gabapentin concentration vs time (Figure 2).

Table 1 Mean clinical laboratory data of 20 subjects

Parameters	Normal values	Mean ± SD	Range
Hemoglobin (g/dl)	12.0 - 18.0	14.99 ± 1.07	13.0 - 17.1
Hematocrit (%)	37.0 - 54.0	43.48 ± 2.88	37.1 - 48.8
Glucose (mg/dl)	70 - 110	84.20 ± 5.84	74.0 - 97.0
BUN (mg/dl)	10 - 20	12.50 ± 2.54	9 - 18
Creatinine (mg/dl)	0.5 - 2.0	1.09 ± 0.14	0.8 - 1.3
SGOT (U/L)	0 - 38	19.70 ± 5.95	12 - 31
SGPT (U/L)	0 - 38	14.80 ± 4.65	7 - 24
Alkaline phosphatase (U/L)	39 - 117	67.70 ± 14.88	49 - 91
Anti HIV	Negative	Negative	Negative
Anti HBsAg	Negative	Negative	Negative
Urinalysis	Normal	Normal	Normal

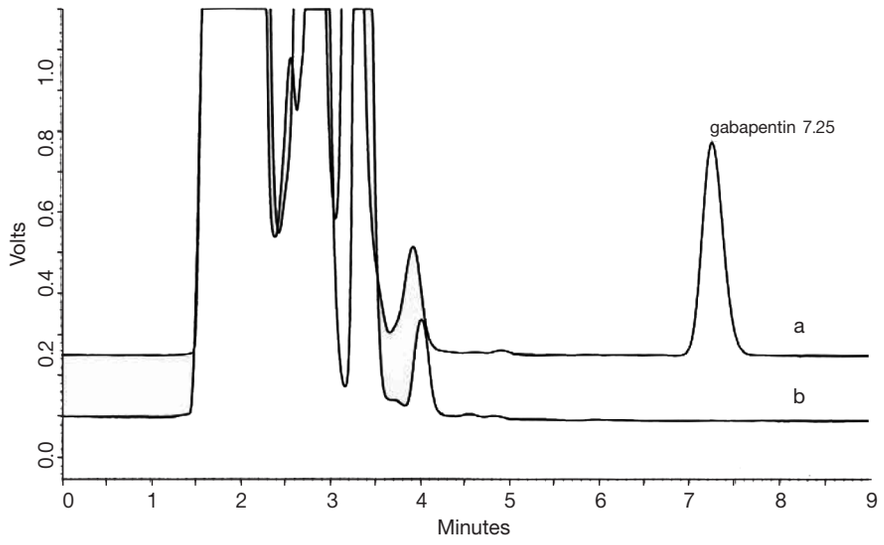

Figure 1 The chromatogram of gabapentin in plasma (a) in comparison to the chromatogram of blank plasma (b).

The relevant pharmacokinetic parameters including peak plasma gabapentin concentrations (C_{max}), time to peak plasma gabapentin concentration (T_{max}), area under the plasma gabapentin concentration-time curve (AUC_{0-t}, AUC_{0-inf}), K_{el} and $T_{1/2}$ are shown in Table 2. T_{max} of Test was 3.08 hours (2-4 hours) and Reference was 3.33 hours (2-5 hours) and the relative ratio was 0.92. By ANOVA and 90% confidence intervals analysis, C_{max}, AUC_{0-t} and AUC_{0-inf} (log transformed data) of Test compared to Reference were presented in Tables 3, 4 and 5, respectively. The result of 90% CI were 96.64-124.94%, 97.60-124.43% and 97.90-124.00% for C_{max}, AUC_{0-t} and AUC_{0-inf}, respectively.

The adverse events were monitored during and after drug administration. All subjects completed the study without any serious adverse events from neither Test nor Reference.

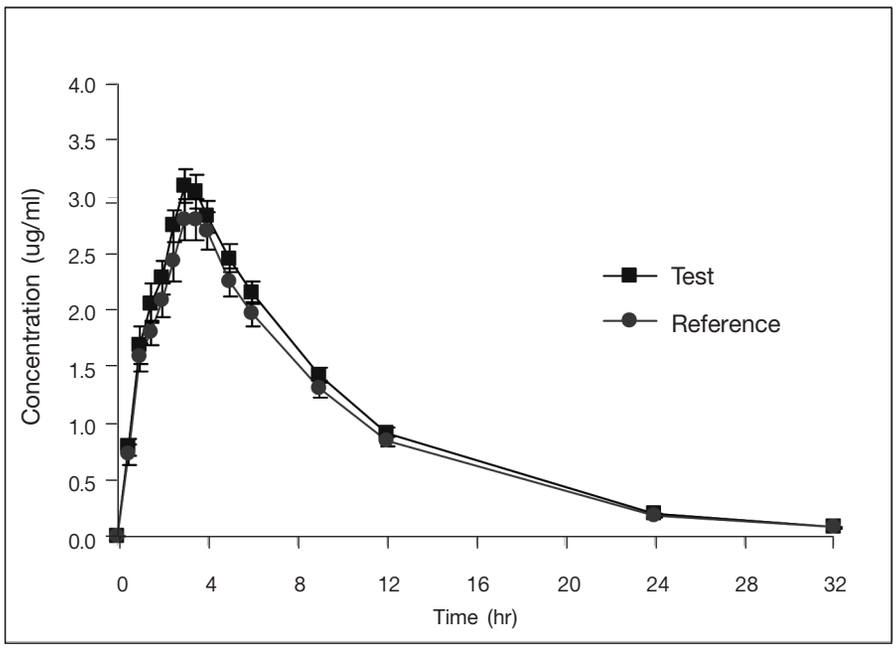

Figure 2 Mean plasma gabapentin concentration-time curve after a single oral dose of 300 mg gabapentin capsule of Test and Reference products (n=20)

Table 2 Mean pharmacokinetic parameters (mean ± SD) of gabapentin from 20 subjects following a single oral dose of 300 mg of Test and Reference products and 90% confidence intervals of those parameters

Parameters	Mean ± SD		90% confidence intervals
	Test	Reference	
C_{max} (mcg/ml)	3.26 ± 0.60	3.00 ± 0.71	96.64-124.94
AUC_{0-t} (mcg.hr/ml)	30.06 ± 4.94	27.63 ± 6.45	97.60-124.43
AUC_{0-inf} (mcg.hr/ml)	30.76 ± 4.88	28.27 ± 6.63	97.90-124.00
T_{max} (hr)	3.08 ± 0.59	3.33 ± 0.63	
$T_{1/2}$ (hr)	5.86 ± 1.06	5.90 ± 0.60	
K_{el} (hr^{-1})	0.12 ± 0.02	0.12 ± 0.01	

Table 3 ANOVA table (logarithmically transformed) and 90% CI of the pharmacokinetic parameter (C_{max})

Source of variance	Degree of freedom	Sum of squares	Mean squares	F	F_{table}
Sequence	1	0.0294	0.0294	2.84	4.35
Drug	1	0.0168	0.0168	1.62	4.35
Period	1	0.0270	0.0270	2.61	4.35
Subject	n-2=18	0.1865	0.0104	1.00	2.12
Error	n-2=18	0.1862	0.0103		
Total	2n-1=39	0.4458			

n= number of subject

	Mean	90% CI
Test	0.50	
Reference	0.46	
Test/Reference	1.09	96.64-124.94

Table 4 ANOVA table (logarithmically transformed) and 90% CI of the pharmacokinetic parameter (AUC_{0-t})

Source of variance	Degree of freedom	Sum of squares	Mean squares	F	F_{table}
Sequence	1	0.0022	0.0021	0.23	4.35
Drug	1	0.0178	0.0178	1.92	4.35
Period	1	0.0137	0.0136	1.48	4.35
Subject	n-2=18	0.2323	0.0129	1.40	2.12
Error	n-2=18	0.1665	0.0093		
Total	2n-1=39	0.4324			

n = number of subject

	Mean	90% CI
Test	1.47	
Reference	1.43	
Test/Reference	1.03	97.60-124.43

Table 5 ANOVA table (logarithmically transformed) and 90% CI of the pharmacokinetic parameter (AUC_{0-inf})

Source of variance	Degree of freedom	Sum of squares	Mean squares	F	F_{table}
Sequence	1	0.0015	0.0015	0.17	4.35
Drug	1	0.0177	0.0177	2.02	4.35
Period	1	0.0122	0.0122	1.39	4.35
Subject	n-2=18	0.2199	0.0122	1.40	2.12
Error	n-2=18	0.1576	0.0088		
Total	2n-1=39	0.4089			

n = number of subject

	Mean	90% CI
Test	1.48	
Reference	1.44	
Test/Reference	1.03	97.90-124.00

Discussion

The purpose of the study was to determine the bioequivalence of gabapentin following administration of 300 mg of test product and reference. The analytical method was modified by the method from the previous methods [3-5] using automated HPLC with fluorescence detection. That was practical and reliable tested by the method validation guidance [5].

Accuracy was presented in term of % recovery. Percent accuracy at low, medium and high concentrations was within the acceptable range 85-115%. In term of precision, the percentage of coefficient of variation in intra-day and inter-day assays were also within the acceptable range (%CV<15%), thus showing validity in accuracy and precision. The standard curve covering the range of human plasma concentration of gabapentin at 300 mg dosage followed good linearity with the correlation coefficient (R^2) close to 1. Gabapentin in plasma was stable within two month long term interval or even three cycle of freeze and thaw.

The mean pharmacokinetic parameters (mean ± SD) of gabapentin from 20 subjects including $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}inf)}$, C_{max} and T_{max} were calculated from the data of plasma gabapentin concentration at each time of blood collection. AUC is the prominent parameter indicating whole drug existing in the body. C_{max} and T_{max} show the evidence involving drug absorption. It was found rapid absorption with time to peak concentration (T_{max}) at 3 hours. The present study also determined the elimination half life of test and reference products. The results are 5.86 and 5.90 hours, respectively. They are not different (p value < 0.05) between the two products. There were previous reports about the pharmacokinetic and route of elimination of gabapentin. Gabapentin is absorbed by an active and saturable transport system, and has a high volume of distribution. It is not bound to plasma proteins, does not induce hepatic enzymes and is not metabolized. At steady state, it has a half-life of 6-8 hours, and is eliminated unchanged by renal route with a plasma clearance proportional to the creatinine clearance [6]. Other investigators reported that half-life of gabapentin was approximately 7 hours necessitates multiple doses daily for many individuals [7]. The other reported the elimination half life of gabapentin was approximately 5 to 9 hours [8] and is about 5 to 7 hours after a single oral dose of 200 to 400 mg [1]. It might be stated that the elimination half life of gabapentin is not different among Thai population and Caucasians.

C_{max}, $AUC_{(0\text{-}t)}$, and $AUC_{(0\text{-}inf)}$ (log transformed data) of test product compared to reference drug were not significantly different when analysed by ANOVA for two way crossover design and 90% confidence interval. 90% CI of C_{max}, $AUC_{(0\text{-}t)}$, and $AUC_{(0\text{-}inf)}$ (log transformed data) of test to reference are within the acceptance criteria of 80-125%. The relative ratio of T_{max} of the test to reference products was 0.92 which was close to 1. These findings show the equivalence of bioavailability of generic and innovator's products. From the ANOVA test for log transformed data of C_{max}, $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}inf)}$, there was no significant difference of sequence, drug, period or subject effects. Finally, it could be concluded that the new generic product of gabapentin and the innovator's product were bioequivalent.

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