Original Article

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Bioequivalence study of torsemide 10 mg tablets in healthy Thai volunteers

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Abstract

Torsemide is a member of high-ceiling loop diuretics which inhibits the $Na^+/K^+/2Cl^-$ symporter in the thick ascending loop of Henle resulting in substantial diuresis and saluresis. Torsemide is indicated for management of edema associated with heart failure and hepatic or renal disease and can be used alone or combined with other antihypertensive drugs in treatment of hypertension. Because there is only one brand of torsemide in Thailand, the Government Pharmaceutical Organization (GPO) has developed a generic product with lower price which would give opportunity for patients to access this medicine. A randomized, open label, two-treatment, two-period, two-sequence, single dose, crossover, bioequivalence study of generic torsemide 10 mg tablets of GPO, Thailand, and the reference product, Unat®, of Roche Farma SA, Spain, in healthy human male and female adult subjects, under fasting conditions was conducted. Washout period was 7 days between treatments. Blood samples were collected at predefined time points up to 24 hours. Plasma concentrations of torsemide were analyzed using liquid chromatography tandem mass spectrometry. Non-compartmental model was used for pharmacokinetic analysis. The 90% CI for the ratios of mean $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max} for the test/reference were 96.5 (93.86-99.29), 96.6 (93.97-99.37) and 91.2 (85.64-97.06), respectively. These values were within the acceptable range of 80.00-125.00. No clinically significant or serious ADRs were observed during the study. Therefore, this study confirmed that Torsemide GPO 10 mg tablets can be used interchangeably to Unat® 10 mg tablets.

Keyword: Torsemide, Pharmacokinetics, Bioequivalence, Liquid chromatography tandem mass spectrometry

1. INTRODUCTION

Torsemide is a member of high-ceiling loop diuretics which inhibits the $Na^+/K^+/2Cl^-$ symporter in the thick ascending loop of Henle resulting in substantial diuresis and saluresis[1]. Its chemical structure is shown in Figure 1. Torsemide is indicated for management of edema associated with heart failure and hepatic or renal disease. It is also can be used alone or combined with other antihypertensive drugs in treatment of hypertension.[2] It is absorbed rapidly after oral administration with a time to maximum concentration within 1 hour.[3] Food has less effect on the oral bioavailability compared with furosemide.[4] Protein binding of torsemide is approximately 97 to 99%[2] and its volume of distribution in healthy volunteers is 0.09 to 0.31 L/kg[3]. However, its tissue distribution is still unclear.[3] 80% of absorbed torsemide is metabolized to several metabolites through hepatic CYP450 enzymes.[2,3] The elimination half-life of torsemide is about 2 to 4 hours and 7 to 8 hours for healthy subjects and cirrhosis patient respectively.[2,5]

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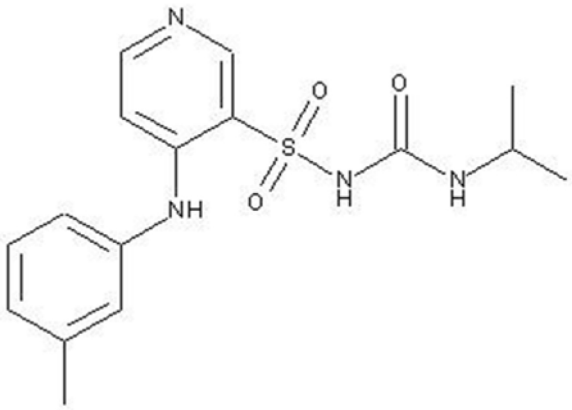

Figure 1. Chemical structure of torsemide

Torsemide is more effective than furosemide for edema caused by heart failure (HF). It also decreases left-ventricular remodeling and mortality in HF patients via its anti-aldosteronergic properties.[4] Because there is only one brand of torsemide in Thailand, the Government Pharmaceutical Organization (GPO) has developed a generic product with lower price which would give opportunity for patients to access this medicine. Therefore, the bioequivalence study is conducted to exhibit the interchangeability between the generic torsemide and the reference product.

2. MATERIALS AND METHODS

2.1. Drug

The test product (Torsemide GPO 10 mg tablets) was manufactured from GPO (Batch number S540221, Manufactured on 25 August 2011, Expiry date 25 August 2013). The reference product (Unat® 10 mg tablets) was manufactured by Roche Farma SA., Spain (Lot number E0042B01, Manufactured on January 2010, Expiry date January 2013).

2.2. Study design

HA randomized, open label, two-treatment, two-period, two-sequence, single dose, crossover, bioequivalence study of generic torsemide 10 mg tablets of GPO, Thailand and the reference product of Roche Farma SA, Spain in healthy human male and female adult subjects, under fasting conditions was conducted. Each subject randomly received a single dose of the assigned formulation in Period I on 18-20 December 2012 and Period II on 25-27 December 21012. Washout period was 7 days between periods. The study protocol was approved by Institute for the Development of Human Research Protections (IHRP).

2.3. Study subjects

26 subjects, 17 males and 9 females, were selected randomly from healthy Thai adult volunteers to participate this study. Subject inclusion criteria included age between 18-55 years with body mass index (BMI) between 18-25 kg/m^2. All subjects were determined healthy judged from medical history, physical examination and laboratory examination (complete blood count, hematocrit, hemoglobin, fasting blood sugar, blood urea nitrogen (BUN), serum creatinine, alkaline phosphatase, ALT, AST, total bilirubin, total protein, albumin, hepatitis B test, urine analysis and ECG). The exclusion criteria included history of hypersensitivity to torsemide or any of excipients, history of illness (like gastrointestinal, hepatic, renal, cardiovascular, diabetes mellitus, and gallstone disease), clinically significant illness within 4 weeks before the start of the study, asthma, urticaria or other allergic type reactions after taking any medication, alcohol dependence, moderate to heavy smoking, consumption of

any medication (including over-the-counter products) for 14 days preceding the study, consumption of tea, coffee or xanthine products more than 3 cups/day within 24 hours prior to the first dose of study, participation in any other clinical trial involving drug administration and collection of blood samples or donation blood in the preceding one month prior to the start of the study. The subjects were informed about risks and benefits of the study and signed informed consent before participating into the study.

2.4. Blood sampling

Blood samples were collected into K_2EDTA vacutainers by the indwelling catheter for eighteen sampling times (0.000, 0.167, 0.333, 0.500, 0.667, 0.833, 1.000, 1.250, 1.500, 1.750, 2.000, 3.000, 4.000, 6.000, 8.000, 10.000, 12.000 and 24.000 hrs). The blood samples were centrifuged at 3,000 ± 100 rcf for 5 minutes at below 10 °C. All plasma samples were transferred to pre-labeled polypropylene tubes and stored at -65 ± 10 °C until analysis.

2.5. Analytical procedure

The plasma concentrations of torsemide in the study samples were quantitated by a validated LC-MS/MS method using torsemide-d7 as the internal standard. The summary of validation results of analyte and internal standard are shown in Table 1. The analyte and internal standard were extracted from plasma using protein precipitation method by acetonitrile (HPLC grade). Then samples were monitored in the positive ion mode by applying ESI using the MRM transitions m/z 349.027→264.050 for analyte and 356.115→264.020 for internal standard, respectively. The concentration of analyte was measured using an 8-point calibration curve. The concentrations of the standards from 4.996 to 5,987.042 ng/mL were calculated using linear regression analysis with a weighing factor of 1/concentration^2. The chromatographic system consisted of ACE 5 Phenyl 150 × 4.6 mm column. The mobile phase was a mixture of 0.2% formic acid solution (v/v) and acetonitrile (30:70%v/v) with a flow rate of 1.0 mL/minute at 40 °C.

Table 1. The summary of validation results

Information requested		**Data**
Linearity (Range)		4.996 to 5,987.042 ng/mL
Selectivity		No interference at the retention time and transition of drug and internal standard.
Coefficient of determination (r^2)		Greater than 0.98
Limit of quantification		4.996 ng/mL
Limit of detection		0.500 ng/mL
Precision	Within-batch (Intra-day precision)	1.3% to 19.7%
	Between-batch (Inter-day precision)	1.7% to 11.9%
Accuracy	Within-batch (Intra-day precision)	97.1% to 104.4%
	Between-batch (Inter-day precision)	99.6% to 102.1%
Recovery of analyte (LQC, MQC and HQC)		96.4%, 100.9% and 101.5%
Recovery of internal standard		100.5%
Matrix effect		No ion suppression or enhancement
Autosampler/Wet extract stability		344.0 hours (within 2 to 8°C)
Freeze and thaw stability		3 cycles
Bench top stability		12.0 hours (at room temperature)
Wet extract bench top stability		6.0 hours (at room temperature)
Reinjection reproducibility		Up to 3rd reinjection

2.6. Pharmacokinetic analysis

The pharmacokinetic parameters ($AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, C_{max}, t_{max}, λ_z and $t_{1/2}$) were determined by non-compartmental model using Phoenix WinNonlin Software Version 6.3. Values below lower limit of quantification (4.996 ng/mL) were set as zero for calculation purposes.

2.7. Statistical analysis

The statistical analysis was conducted using SAS® Version 9.3. The primary pharmacokinetic parameters ($AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max}) were transformed to natural logarithm scale (ln) before statistical analysis. Bioequivalence of test product-T vs. reference product-R was concluded, if the 90% confidence interval of ratio of geometric least square mean fell within the acceptance range of 80.00-125.00% for ln-transformed pharmacokinetic parameters $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max} of torsemide.

3. RESULTS AND DISCUSSION

26 Subjects, 17 males and 9 females, were enrolled to the study with an average age of 30.31±7.39 years and a mean BMI of 21.58±1.66 kg/m^2. All enrolled subjects were used for pharmacokinetic and statistical analyses. Both products were well tolerated and no serious ADR observed. There was only one adverse event observed from dizziness and hypotension symptom.

The mean plasma concentration versus time profile of torsemide is illustrated in Table 2 and Figure 2. The pharmacokinetic parameters ($AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, C_{max}, t_{max}, λ_z and $t_{1/2}$) of the test and reference products are summarized in Table 3. ANOVA model included Sequence, Formulation and Period as fixed effects. An F-test was performed to determine the statistical significance of the effects at a significance level of 5% (α=0.05) are showed in Table 4. The 90% confidence intervals for ln-transformed $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max} were within the bioequivalence range of 80.00 – 125.00% as represented in Table 5. These results revealed that the test product and the reference product were bioequivalent. The power of all primary pharmacokinetic parameters ($AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$ and C_{max}) were which were greater than 80% indicating that the number of subjects was enough to confirm the bioequivalence of two formulations.

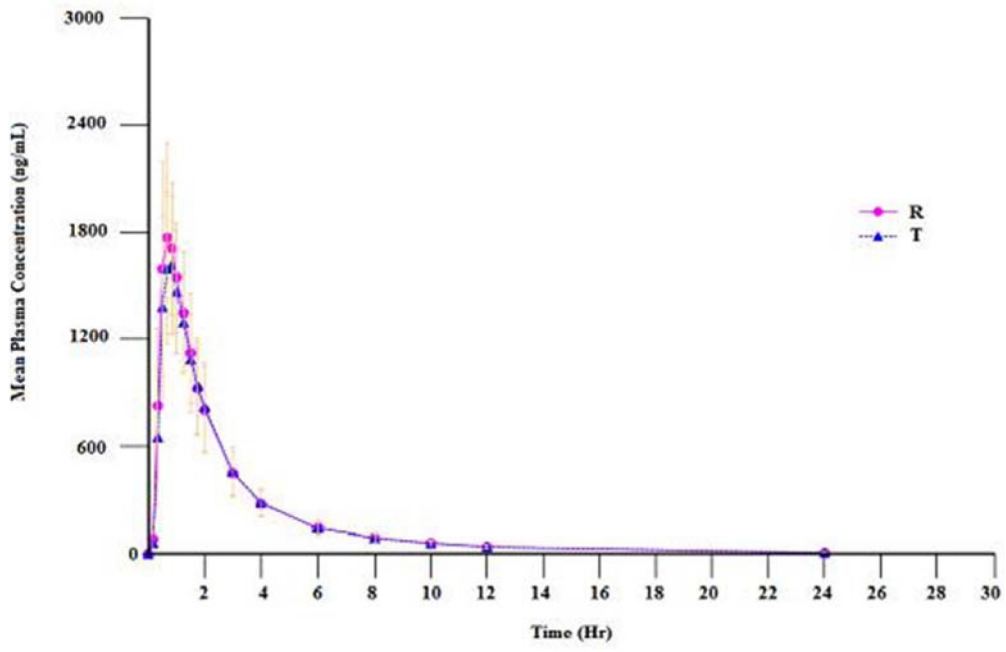

Figure 2. Linear plot of mean (±SD) plasma concentration of torsemide versus time curves after oral administration of test product-T and reference product-R in healthy Thai volunteers under fasting conditions (N=26)

Table 2. The mean (±SD) plasma concentration of torsemide versus time after oral administration of test and referent products (N=26)

Time (hr)	Mean (±SD) plasma concentration for test product (ng/mL)	Mean (±SD) plasma concentration for reference product (ng/mL)
0.000	0.000 ± 0.0000	0.000 ± 0.0000
0.167	56.069 ± 62.1387	82.001 ± 80.8198
0.333	647.913 ± 395.9378	825.669 ± 433.2924
0.500	1,377.223 ± 509.9929	1,593.208 ± 604.1650
0.667	1,594.713 ± 425.5812	1,770.048 ± 539.5800
0.833	1,614.656 ±384.8760	1,709.574 ± 371.9008
1.000	1,464.755 ± 345.9971	1,546.841 ± 304.7809
1.250	1,295.197 ± 275.6373	1,345.552 ± 343.2460
1.500	1,085.958 ± 247.3208	1,121.878 ± 333.9679
1.750	938.514 ± 269.4127	921.967 ± 267.3365
2.000	818.384 ± 247.6067	800.049 ± 243.7416
3.000	451.973 ± 138.5812	445.804 ± 119.4721
4.000	283.405 ± 72.5119	277.893 ± 72.9615
6.000	140.306 ± 33.9343	142.385 ± 42.2576
8.000	86.699 ± 21.3750	88.658 ± 24.5382
10.000	57.890 ± 14.1927	61.008 ± 17.8773
12.000	38.088 ± 10.6585	39.260 ± 12.5532
24.000	5.294 ± 3.8507	6.032 ± 4.5859

Table 3. The pharmacokinetic parameters of the test and reference products

Product	$AUC_{0\text{-tlast}}$ (ng.hr/mL)	$AUC_{0\text{-}\infty}$ (ng.hr/mL)	C_{max} (ng/mL)	t_{max} (hr)	λ_z (1/hr)	$t_{1/2}$ (hr)
Test	4,222.585 ± 747.4567	4,296.891 ± 731.1465	1,802.695 ± 335.6063	0.750 (0.500-2.000)	0.173 ± 0.0368	4.172 ± 0.7696
Reference	4,379.601 ± 820.9318	4,453.685 ± 813.2174	1,977.267 ± 390.3282	0.667 (0.500-1.500)	0.170 ± 0.0360	4.231 ± 0.7986

Table 4. The ANOVA of pharmacokinetic parameters of torsemide for ln-transformed data (N=26)

Source	p-values		
	$AUC_{0\text{-tlast}}$	$AUC_{0\text{-}\infty}$	C_{max}
Period	0.1146	0.1211	0.3394
Treatment	0.0423	0.0468	0.0186
Sequence	0.1295	0.1226	0.3549

Table 5. 90% confident intervals of the ln-transformed primary pharmacokinetic parameters

Parameters	Ratio	90% CI
$AUC_{0\text{-}tlast}$	96.5	93.86-99.29
$AUC_{0\text{-}\infty}$	96.6	93.97-99.37
C_{max}	91.2	85.64-97.06

4. CONCLUSION

The test product, Torsemide GPO 10 mg tablets, met the criteria of 80.00-125.00% for bioequivalence with respect to the rate and extent of absorption when compared with the reference product, Unat® 10 mg tablets. Both products were well tolerated. Therefore, this study confirmed that Torsemide GPO 10 mg tablets can be used interchangeably to Unat® 10 mg tablets.

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