Bioequivalence Study of 100 mcg Levothyroxine Sodium Tablets in Patients Who Have Hypothyroidism with Euthyroid in Steady State

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ABSTRACT

Objective: The purpose of this study was to ascertain the bioequivalence between a test product (100 mcg levothyroxine sodium tablets; Thyrosit®, SRIPRASIT PHARMA Co., Ltd., Thailand) and the reference product (100 mcg anhydrous thyroxine sodium; Eltroxin®, Glaxo SmithKline).

Methods: An open-label, 2-treatment, 2-period, 2-sequence, randomized crossover design without washout period was studied in 16 patients (8 females in group 1, 1 male and 7 females in group 2) who were diagnosed hypothyroidism with euthyroid in steady state. The enrolled subjects were given a tablet of 100 mcg levothyroxine sodium either the test or reference product daily for 57 days of each period. At steady state (Day 57 on period 1 and Day 114 on period 2), blood samples were collected over a 24-h interval and the concentrations of T4 were examined by a Microparticle Enzyme Immunoassay technique. Pharmacokinetic parameters were evaluated from plasma concentration-time profiles of T4 using the non-compartmental analysis without the adjustment of baseline levels.

Results: The 90% confidence interval of geometric mean ratio of primary target parameters (C^{ss}_{max} and $AUC_{0\text{-}24(ss)}$) between the test and reference formulations was entirely within the bioequivalence acceptance limits of 80.00-125.00%, which was 103.32% (97.99-108.93%) for C^{ss}_{max} ratios, and 102.32% (96.97-107.96%) for $AUC_{0\text{-}24(ss)}$) ratios, together with the power more than 80%. In addition, the nonparametric Friedman's test for T^{ss}_{max} demonstrated no significant difference between the two formulations ($p>0.05$). All subjects tolerated their medication well. No serious adverse effect was observed.

Conclusion: The test and reference products of thyroxine in this study are bioequivalent and well tolerated.

Keywords: Levothyroxine, hypothyroidism, bioequivalence

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INTRODUCTION

Hypothyroidism is a common endocrine disorder resulting from thyroid hormone deficiency. It could be found mostly as sequelae after thyroidectomy or treatment with I-131 therapy or antithyroid drug such as propylthiouracil. Levothyroxine sodium is the monosodium salt of the laevorotatory isomer of thyroxine (tetraiodothyronine; T4), an essential hormone in energy metabolism obtained from the follicles of the thyroid gland. Levothyroxine sodium products are used to treat simple goiter and hypothyroidism. Patients with hypothyroidism usually receive lifelong levothyroxine therapy.[1]

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The oral bioavailability of levothyroxine ranges from 40 to 80%. This drug is mostly absorbed from the jejunum and upper ileum. More than 90% of levothyroxine in plasma is bound to proteins especially thyroxine binding globulin (TBG), but only free (unbound) hormone is pharmacologically active. The main metabolic pathway of levothyroxine is deiodination occurring in liver, kidneys and myocardium. It is also metabolized via phase II reactions such as conjugation with glucuronide and sulfate group; the metabolites are then excreted directly into the bile and small intestine with enterohepatic circulation. In normal conditions, the elimination half-life of levothyroxine is 6-7 days.[2]

Levothyroxine sodium has a narrow therapeutic index. Dosing adjustment of this drug dosage should be based primarily on the clinical response and reassured by thyroid function test.[1] Thus, assessment of potency and bioavailability of the various marketed levothyroxine products are crucial.[3-9]

Results from previous studies regarding the bioequivalence of the 4 generic and brand-name levothyroxine preparations were different, but were bioequivalent by Food and Drug Administration criteria and were interchangeable in the majority of patients receiving thyroxine replacement therapy.[5-6]

The bioequivalence study of two products of 100 mcg levothyroxine was evaluated. This study will ascertain a comparison between the test and the reference products when prescribing the less expensive generic drug.

MATERIALS & METHODS

Study drugs

Thyrosit® offered by SRIPRASIT PHARMA Co., Ltd., Thailand and Eltroxin® from GlaxoSmith Kline were used as test and reference products, respectively. Both products were tablets containing 100 mcg of levothyroxine sodium.

Subjects

Sixteen patients with primary hypothyroidism, both male and female, aged between 20-70 years participated in this study. Clinical and laboratory evidence were used to confirm the euthyroid status of the patients. They took 100 mcg of levothyroxine sodium once a day for a minimum of 3 months prior to enrollment in the study. They had normal or acceptable laboratory data [electrocardiogram (ECG), serum cortisol level, thyroid function test, fasting plasma glucose (FPG), triglyceride, cholesterol, complete blood count (CBC), liver function test, renal function test, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), follicle stimulating hormone (FSH) and urinalysis]. Pregnant or lactating women were ineligible for enrollment.[10-11]

Study design

An open-label, randomized, multiple dose, 2-treatment, 2-period, 2-sequence crossover design without washout period was used in this study. Written informed consent was done prior to performing study procedures. Eligible patients who enrolled into this study were divided into 2 groups. They were given 100 mcg of Thyrosit® and Eltroxin®; whichever was given first was determined by randomization and then changed to another product at day 57. The subjects who received Thyrosit® (test product) in period I and the Eltroxin® (reference product) in period II were grouped into group 1 (TR group) while the subjects who received the reference product in period I followed by the test product in period II were grouped into group 2 (RT group). All subjects took a tablet of 100 mcg of levothyroxine for at least 2 hours after meal and at the same time every day with no washout period. The adherence to medication was checked by counting the pills and rates of prescription refills, interviewing the patients and observing the clinical outcomes. All subjects were given each product (test and reference) for 57 days and were hospitalized 2 times at day 57 and 114 for assessing the pharmacokinetic parameters of T4 in the serum.[11]

Measuring the serum levels of total thyroxine (T4) was done before the trials as pre-dose values and on study days 21, 42, 78 and 99. After the last dose of test and reference products on days 57 and 114, serum T4 levels were measured again to achieve the time profile at the following scheduled times. Then, each subject returned to the

clinical site and stayed overnight for the following bioequivalence study. Prior to the admission day, all subjects fasted for a minimum of 8 hours beginning at midnight. With the exception of 240 mL of water given with study drug, no food or liquid was allowed for at least 4 hours after dosing. Blood samples were collected in red-top vacutainer tubes at time 0 (pre-dose sample) and at 1, 2, 3, 4, 6, 8, 10 and 24 hours after levothyroxine was orally taken. Serum levels of T4 were measured by a Microparticle Enzyme Immunoassay (MEIA) technique using commercial kits (Abbott Laboratories, USA).[11] Tolerability was assessed from objective and subjective observations of vital signs and adverse events.

The clinical part was conducted at Siriraj Clinical Research Center, Faculty of Medicine Siriraj Hospital, Mahidol University. The study protocol was approved by the Ethics Committee of Faculty of Medicine Siriraj Hospital. The study was performed in accordance with the Declaration of Helsinki for biomedical research involving human subjects and the Guideline for Good Clinical Practice.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters including the maximum observed plasma concentration at steady state (C^{ss}_{max}), the lowest observed plasma concentration at steady state (C^{ss}_{max}), the average plasma concentration at steady state (C^{ss}_{av}), the observed plasma concentration at 24 h of dosing (C^{ss}_{24}), the area under the plasma concentration-time curves at steady state ($AUC_{0\text{-}24(ss)}$), the time taken to peak concentration at steady state (T^{ss}_{max}), the plasma elimination half-life ($T_{1/2}$) and the terminal rate constant (λz) were obtained using a non-compartmental analysis method by WinNonlin®, version 3.1, without the adjustment of baseline levels since endogenous levothyroxine concentrations were unpredictable during the course of the study. These parameters were subjected to a comparative statistical analysis by determining the position of the 90% confidence intervals for the individual ratios "test/reference" by least square means of ANOVA of logarithmically transformed data.

TABLE 1. Demographic data and baseline characteristics of subjects.

Characteristics		Group 1 (TR group) (n=8)	Group 2 (RT group) (n=8)
Gender	Male	0	1
	Female	8	7
Age (years)		49±11.40	39±11.73
Weight (kg)		56.8±8.23	58.1±7.58
Height (cm)		156.6±4.23	158.4±8.45
Body mass index (kg/m^2)		23.12±2.87	23.10±1.90
Vital signs	Temperature (°C)	36.7±0.2	36.7±0.2
	Pulse (beats/minute)	72±11.96	72±7.92
	Respiratory rate (times/minute)	21±1.41	20±1.28
	Systolic blood pressure (mmHg)	124±16.45	115±14.97
	Diastolic blood pressure (mmHg)	79±7.07	72±10.78

TR group (group 1) = subjects who received test product in period I and the reference product in period II
RT group (group 2) = subjects who received the reference product in period I followed by the test product in period II

RESULTS

Demographic data

Demographic characteristics of enrolled subjects have been shown in Table 1. The TR group consisted of 8 females with mean age and body mass index (BMI) of 49 years, and 23.12 kg/m^2, respectively. The RT group consisted of 7 females and 1 male with mean age of 39 years and BMI of 23.10 kg/m^2.

Bioavailability and pharmacokinetic parameters

The C^{ss}_{max} and $AUC_{0\text{-}24(ss)}$ values received from the test product were approximate to those obtained from the reference product as shown in Table 2. The geometric mean C^{ss}_{max} of the reference and test products was 11.2 and 11.5 mcg/dL, respectively while the geometric mean $AUC_{0\text{-}24(ss)}$ for the reference and test products was 233 mcg·hr/dL and 238 mcg·hr/dL, respectively. The geometric mean C^{ss}_{min}, C^{ss}_{24} and Cssav for the test product were 8.78 mcg/dL, 9.40 mcg/dL and 9.93 mcg/dL, respectively, which were insignificantly higher than those for the reference product, which were 8.66 mcg/dL, 9.28 mcg/dL and 9.71 mcg/dL, respectively. Furthermore, the percentage fluctuation at steady state was 23.9% and 24.8% for the reference and the test products, respectively, which was considered very low. The median (range) of the time to achieve the maximum concentration at steady state (T^{ss}_{max}) of T4 of 3.00 (0.00-4.00) h for the test product was found to be greater than that of 1.00 (0.00-4.00) h for the reference product.

The statistical analysis from this study revealed that the point estimate (90% confidence interval) of the geometric mean ratio (test/reference) of the primary parameters; C^{ss}_{max} and $AUC_{0\text{-}24(ss)}$ were within the equivalence criteria of 80.00-125.00% which were 103.32% (97.99-108.93%) for C^{ss}_{max} ratios and 102.32% (96.97-107.96%) for $AUC_{0\text{-}24(ss)}$ ratios. In addition, all geometric

TABLE 2. Pharmacokinetic parameters of the test (Thyrosit®) and reference (Eltroxin®) products with 90% CI of the geometric mean ratios (T/R).

Pharmacokinetic parameters	Test product (Thyrosit®)	Reference product (Eltroxin®)	90% CI of the geometric mean ratios (T/R)
C^{ss}_{max} (mcg/dL)	11.5	11.2	97.99 - 108.93
$AUC_{0\text{-}24(ss)}$ (mcg·hr/dL)	238	233	96.97 - 107.96
C^{ss}_{min} (mcg/dL)	8.78	8.66	95.80 - 107.30
C^{ss}_{24} (mcg/dL)	9.40	9.28	95.47 - 107.39
C^{ss}_{av} (mcg/dL)	9.93	9.71	96.97 - 107.96
T^{ss}_{max} (h)	3	1	-

C^{ss}_{max} = Plasma concentration at steady state, $AUC_{0\text{-}24(ss)}$ = Area under the plasma concentration-time curve at steady state, C^{ss}_{min} = Lowest observed plasma concentration at steady state, C^{ss}_{24} = Observed plasma concentration at 24 h of dosing, C^{ss}_{av} = Average plasma concentration at steady state, T^{ss}_{max} = Time taken to peak concentration at steady state

TABLE 3. Statistical summary of the comparative bioavailability data (N=16).

Dependent	Geometric mean ratio (T/R)	90% CI Lower limit	90% CI Upper limit	Power
Ln (C^{ss}_{max})	103.32	97.99	108.93	1.000
Ln ($AUC_{0\text{-}24(ss)}$)	102.32	96.97	107.96	1.000
Ln (C^{ss}_{min})	101.39	95.80	107.30	0.999
Ln (C^{ss}_{24})	101.26	95.47	107.39	0.999
Ln (C^{ss}_{av})	102.32	96.97	107.96	1.000

T = Test product R = Reference product

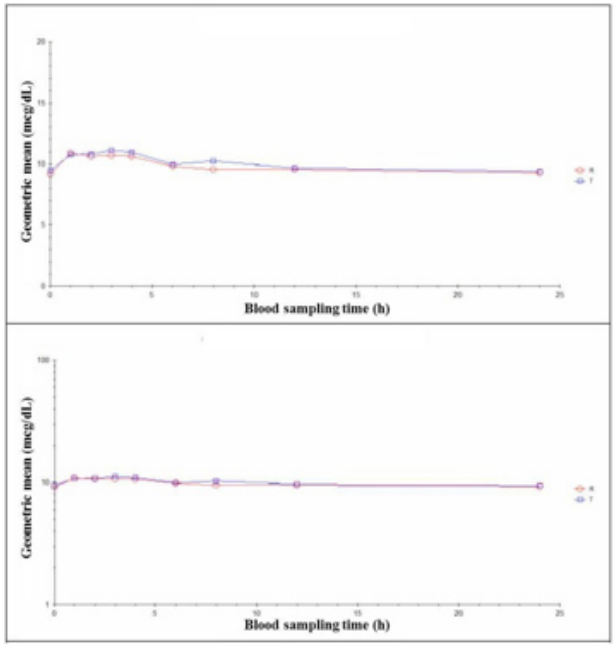

Fig 1. Geometric mean of plasma concentration-time profile of T4 (N=16); normal plot (above) and semilog plot (below).

mean ratios of C^{ss}_{min}, C^{ss}_{24} and Cssav between test and reference products were correlated within the bioequivalence acceptance limits with the power of test of more than 80% as shown in Table 3 and Fig 1.

Regarding to the secondary parameter T^{ss}_{max}, Chi-square test, which is a nonparametric statistical method, was used to determine the difference in median Tssmax values between the two products using Friedman's test. WinNonlin®,version 3.1, and Kinetica 2000 software were used to analyze the untransformed T^{ss}_{max} data. The conclusion from analysis revealed that there was no statistical difference of median Tssmax between test and reference products ($p>0.05$).

Tolerability assessment

Concerning to the patients' safety, surveillance of adverse events was done throughout the study. Vital signs were assessed at screening and during the entire study period. No abnormalities were detected in terms of body temperature, blood pressure, heart rate, and respiratory rate. Additionally, no abnormalities were shown on ECG. Blood chemistry tests, including creatinine, cholesterol, LDH, and CPK were measured at screening and after study drug dosing of both test and reference products. These laboratory values were within acceptable limits and indicated no clinically significant change. No serious adverse events were observed throughout this study. The minor adverse events including headache, syncope, common cold (or respiratory tract infection), laryngitis, knee pain, ankle pain, constipation and diarrhea which were detected in 8 subjects. All of these events were considered unrelated to the study drugs.[2] However, all adverse events during the study were reported to the Ethics Committee of Faculty of Medicine Siriraj Hospital, Mahidol University.

DISCUSSION

The clinical use of levothyroxine requires careful titration and close monitoring because it has a narrow therapeutic index. A drug product with lesser potency or bioavailability can lead to a suboptimal response and subsequently a subtherapeutic effect. On the contrary, substitution of a drug product with greater potency or bioavailability will result in symptoms of overt hyperthyroidism such as chest pain, palpitation or cardiac arrhythmia.[1] Thus, there are various guidance and literature reviews recommending bioequivalence studies of levothyroxine.[3-6] In Thailand, the patients with hypothyroidism have usually been treated with lower dose (e.g., 50-100 mcg of levothyroxine) which may be correlated with the body weight.[12] With this clinical data, we chose the 100 mcg levothyroxine in this bioequivalence study. There are many products of levothyroxine, including original and generic drugs, available in Thailand. Results of this study would ensure doctors and patients understand the use of a generic drug of levothyroxine in the treatment of primary hypothyroidism.

The data from this study revealed statistical equivalence for the essential pharmacokinetic parameters including C^{ss}_{max}, C^{ss}_{av}, C^{ss}_{24}, Cssmin, and $AUC_{0\text{-}24(ss)}$ of the test and reference products. The 90% confidence intervals of these parameters were within the limits (80.00-125.00%) and can be accepted by any regulatory agency. The power of all parameters was above 80% indicating that a sample size of 16 patients was adequate. In addi-

tion, nonparametric Friedman's test for T^{ss}_{max} was performed to demonstrate no significant difference between the two formulations ($p>0.05$). Finally, it can be concluded that the test (Thyrosit®) and reference (Eltroxin®) products of 100 mcg of levothyroxine are bioequivalent.

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