Bioequivalence of Two Levothyroxine Tablet Formulations Without and With Mathematical Adjustment for Basal Thyroxine Levels in Healthy Argentinian Volunteers: A Single-Dose, Randomized, Open-Label, Crossover Study

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ABSTRACT

Background: Levothyroxine has a narrow therapeutic index; therefore, precise and accurate assessment of the bioequivalence of different levothyroxine products is critical. Bioavailability estimates of levothyroxine formulations might be affected by baseline concentrations of the hormone.

Objectives: The aim of this study was to assess the bioequivalence of 100 μg of a test (T4 Montpellier® 100, Química Montpellier S.A., Buenos Aires, Argentina) and reference (Synthroid®, Abbott Laboratories, Abbott Park, Illinois) formulation of levothyroxine. We also compared 2 methods of levothyroxine measurements: without and with baseline correction for endogenous levothyroxine.

Methods: This randomized, open-label, 2-sequence, crossover study with a 65-day washout period was carried out in healthy, white, euthyroid volunteers following a single dose of sodium levothyroxine 600 μg. Blood samples were collected at 30 and 15 minutes prior to administration, and 0 (baseline), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, and 48 hours to determine thyroxine; serum thyrotropin (TSH) concentrations were determined 30 minutes before administration and 48 hours after administration. Serum concentrations of thyroxine were determined through radioimmunoassay and serum TSH concentrations were determined by a validated 2-site immunoradiometric assay. The formulations are considered to be equivalent if the 90% CI ratios for C_{max} and AUC_{0-last} are within 80% to 125%, per the US Food and Drug Administration (FDA). Adverse event monitoring was performed throughout the study by assessing clinical parameters (eg, blood pressure, electrocardiogram) and patient reports.

Results: A total of 24 volunteers (16 male, 8 female; mean [SD] age, 30.2 [4.6] years [range, 21–40 years]; mean [SD] weight, 71.71 [7.52] kg [range, 58–83 kg]) were included in the study. Without adjustment for baseline levels of endogenous levothyroxine, geometric mean C_{max} for the test and reference formulations were 8.92 and 9.39 μg/dL, respectively; AUC_{0-last} values were 368.40 and 383.37 μg/mL · h^{-1}. The 90% CI of the geometric mean for the percent ratios (test:reference) of C_{max} and AUC_{0-last} were 95.1% (90% CI, 91.9–98.3) and 96.1% (90% CI, 94.0–98.2), respectively. With adjustment for baseline levels of endogenous levothyroxine, the geometric mean C_{max} for the test and reference formulations were 3.16 and 3.39 μg/dL, respectively; AUC_{0-last} values were 88.33 and 95.60 μg/mL · h^{-1}. Despite performing the adjustment, the 90% CI of the geometric mean for C_{max} and AUC_{0-last} test:reference ratios were 93.1% (90% CI, 84.9–102.2) and 92.4% (90% CI, 85.2–100.2), respectively. No significant between-

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group differences were found with regard to pharmacokinetic parameters. No adverse events were observed or reported.

Conclusion: The results of this study suggest that the test formulation was bioequivalent to the reference formulation of levothyroxine in these healthy volunteers, according to the US FDA definition of bioequivalence. This was supported by the analysis of concentration–time profiles without and with correction for basal endogenous levothyroxine. (*Clin Ther.* 2008;30:2015–2023) © 2008 Excerpta Medica Inc.

Key words: levothyroxine, thyroxine, bioequivalence, pharmacokinetics, methodology, endogenous.

INTRODUCTION

Levothyroxine, an endogenous hormone secreted by the thyroid, is subject to a complex biologic regulation. Levothyroxine has 2 characteristics that make the determination of bioequivalence among thyroxine (T_4) products challenging. First, the synthetic compound levothyroxine sodium is biochemically and physiologically indistinguishable from endogenously produced levothyroxine.[1] Second, levothyroxine has a narrow therapeutic range (NTR) with the potential for iatrogenic hyperthyroidism or hypothyroidism at doses 25% lower or greater than what is considered optimal, based on patients' serum thyrotropin (TSH) concentration.[2] On the same grounds, data from large public health surveys[3,4] and studies of general clinical practice[5] suggest that 15% to 29% of patients receive insufficient doses of levothyroxine, whereas 18% to 24% receive excessive doses. The bioavailability and bioequivalence between the different marketed levothyroxine formulations have been recognized as important issues in current guidelines.[6,7] To measure the bioavailability of levothyroxine formulations, the US Food and Drug Administration (FDA)[8] recommends administering a single dose (600 μg), several times above the typical therapeutic dose, to healthy volunteers with sample collection to 48 hours. The theory is to raise serum concentrations of the hormone high enough above the endogenous baseline levels of levothyroxine to achieve meaningful pharmacokinetic measurements. Bioavailability studies in healthy subjects have found that, even with such doses, endogenous levothyroxine contributes to the total AUC. Nonetheless, the FDA[8] recommended that pharmacokinetic profiles to assess bioequivalence should be presented without adjustment by baseline levels because endogenous levothyroxine concentrations are unpredictable during the course of the study. Controversy arose after the publication of a report by Blakesley et al,[9] suggesting that the application of criteria for bioequivalence without taking into account endogenous levothyroxine levels resulted in a failure to identify differences by as much as 25% to 33% in dose strength between products. Interestingly, corrections to compensate for endogenous levothyroxine reduced the chances of 2 levothyroxine products being declared bioequivalent when they differed by 25%.[10] However, the use of correction methods did not eliminate the chance that 2 products differing by 12.5% would be declared bioequivalent.[10,11]

FDA guidelines also state that washout periods of ≥35 days would be good enough to prevent carryover effects from one administration period to the next. However, Blakesley et al[9] found significant carryover effects with washout periods >45 days.

To comply with the regulatory requirements of South Africa, the relative bioavailability of a levothyroxine drug product, already marketed in Argentina, had to be compared with an innovator product. Therefore, the main objective of this study was to assess the bioequivalence of 2 levothyroxine sodium 100-μg tablet formulations (T4 Montpellier® 100, Química Montpellier S.A., Buenos Aires, Argentina, test formulation; Synthroid®, Abbott Laboratories, Abbott Park, Illinois, reference formulation) following a 2 × 2 crossover design with a 65-day washout period in healthy euthyroid volunteers. The data obtained allowed for the analysis of potential influence from endogenous basal concentrations of levothyroxine through the introduction of a mathematical adjustment.

SUBJECTS AND METHODS

Subjects

Healthy Argentinian volunteers were enrolled in the study. Exclusion criteria included history of cardiovascular, hepatic, renal, psychiatric, neurologic, hematologic, and metabolic disease; drug or alcohol abuse within 2 years of study initiation; smoking; HIV; hepatitis B or C virus; consumption of other prescribed or over-the-counter drugs within 2 weeks before the study; or participation in a similar study within the past 6 months. These criteria were confirmed through blood testing and patient report.

The protocol and informed consent were approved by the ethics committee and the institutional review board of the Hospital de Clínicas José de San Martín, Universidad de Buenos Aires, and by Argentina's National Regulatory Agency (ANMAT), Buenos Aires, Argentina. The study was performed according to the revised Declaration of Helsinki (Tokyo 2004) for biomedical research involving human subjects and the principles of Good Clinical Practices.

After signing informed consent, patients underwent clinical examination; electrocardiogram, chest radiograph, and routine clinical chemistry were performed 2 weeks before hospitalization.

Laboratory analyses consisted of blood hemoglobin; hematocrit; total white blood cell count using an automatic blood cell counter (Roche Micros Analyzer, Micros OT, Roche Diagnostics Inc., Somerville, New Jersey) and differential white blood cell count using light microscopy. Blood glucose, triglycerides, and total cholesterol analyses were performed with an enzymatic method; albumin, direct and indirect bilirubin by a colorimetric method; creatinine, aminotransferases (aspartate aminotransferase, alanine aminotransferase), and γ-glutamyl transpeptidase using a kinetic method, as recomended by the manufacturer. All were performed in an autoanalyzer (Express Plus, Ciba Corning Diagnostics Corporation, Medfield, Massachusetts). HIV and hepatitis B virus antibodies were analyzed using a commercial agglutination kit (Fujirebio Inc., Tokyo, Japan); hepatitic C virus antibodies were determined using a solid-phase enzyme-linked immunosorbent assay; antimicrosomal and antithyroglobulin antibodies using agglutination; pregnancy test using immunoradiometric assay and urinalysis; and urinary sediment using reactive strips and microscopy. Female subjects were not to be pregnant, expecting to get pregnant, or breastfeeding at the time of the study and were required to be using an effective contraception method (intrauterine device or hormonal method) throughout the study.

Study Design

This was a randomized, open-label, crossover study, with a 65-day washout between periods, conducted from April 2006 to October 2006. Volunteers were admitted to the hospital the night before drug administration. After an overnight fast, blood samples (10 mL) from a suitable antecubital vein were collected into sodium heparin (20:1)–containing tubes at 30 and 15 minutes prior to administration, and 0 (baseline), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, and 48 hours after administration of a single oral dose of sodium levothyroxine 600 μg (six 100-μg tablets) of either the test or reference formulation with 240 mL of water. TSH was determined 30 minutes before administration and 48 hours after administration. After centrifuging at 2500*g* for 20 minutes at room temperature (~22°C), plasma was separated, aliquoted, and stored at –20°C until quantification.

Adverse event monitoring was performed throughout the study by assessing clinical parameters (eg, blood pressure, electrocardiogram) and patient reports.

Levothyroxine and Thyroid-Stimulating–Hormone Assay

Serum concentrations of T_4 were determined through the use of a commercial radioimmunoassay method (Coat-A-Count, Diagnostics Product Corporation, Los Angeles, California). Serum TSH concentrations were determined by a validated 2-site immunoradiometric assay (TSH IRMA, Diagnostics Product Corporation, Los Angeles, California). According to the kit insert, normal range is 0.30 to 5.00 mUI/mL.[12]

The assays were specifically validated for this study over the following concentration ranges: 1.00 to 30.0 μg/dL for T_4 and 0.250 to 52.0 mUI/mL for TSH. All calibration curves had correlation coefficients (r) ≥ 0.9978. The average back-calculated calibration standards had CVs between 1.25% and 2.60%. The interassay CV of the quality controls (theoretical concentrations of 2.00, 6.00, and 18.0 μm/dL) from the analytical runs ranged from 7.4% to 13.8%, with percent differences from theoretical ranging from 0.0295 to 0.800. Analytes from each subject for all dosing regimens were measured in duplicate in the same analytical assay.

Pharmacokinetic and Statistical Analysis

Levothyroxine plasma profiles and pharmacokinetic measures were analyzed without and with adjustment by baseline levels of endogenous T_4. For each subject and period, the mean of the 3 levothyroxine values at 30 and 15 minutes before administration and baseline were subtracted from each T_4 concentration after administration.

Levothyroxine concentrations were analyzed as a function of time and the following pharmaco-

kinetic parameters were obtained for each formulation: C_{max}, T_{max}, and AUC_{0-48h}. AUC_{0-48h} was calculated by applying the linear trapezoid rule. Values for T_{max} were compared using the Wilcoxon signed rank test.[13]

Mean values for C_{max} and AUC_{0-48h} were adjusted for body weight by using a multivariate analysis of covariance (MANCOVA) model.

Pharmacokinetic parameters applying a noncompartmental model (C_{max} and AUC_{0-48h}) of levothyroxine data were log transformed and compared by analysis of variance (ANOVA) for a crossover design, taking into account the effect of formulations, periods, sequences, and subjects on these parameters.[14,15]

To set the limits of bioequivalence, the tests of Schuirmann[16] and Anderson and Hauck[17] were used. Differences were considered significant when $P < 0.05$.

The ratios and 90% CIs of C_{max} and AUC_{0-48h} for both formulations were calculated and 2 one-sided *t* tests[14] were employed to assess the confidence limits were within the US FDA criteria for bioequivalence (80%–125%).[8,15,18]

TSH values at 30 minutes before administration compared with those determined at 48 hours after administration were analyzed by ANOVA of the log-transformed values. All pharmacokinetic and statistical analyses were carried out with WinNonlin Professional software version 5.0 (Pharsight Corporation, Palo Alto, California).

RESULTS

A total of 24 volunteers (16 male, 8 female; mean [SD] age, 30.2 [4.6] years [range, 21–40 years]; mean [SD] weight, 71.71 [7.52] kg [range, 58–83 kg]) participated in the study.

Both formulations were associated with a significant increase ($P < 0.05$) in levothyroxine concentration, allowing for pharmacokinetic analysis. Mean levothyroxine plasma concentrations versus time without and with adjustment of baseline levels of endogenous T_4 are shown in **Figures 1** and **2**, respectively.

Table I shows levothyroxine pharmacokinetic parameters derived for each of the treatments without and with correction for baseline levothyroxine concentrations, and the geometric means and 90% CI val-

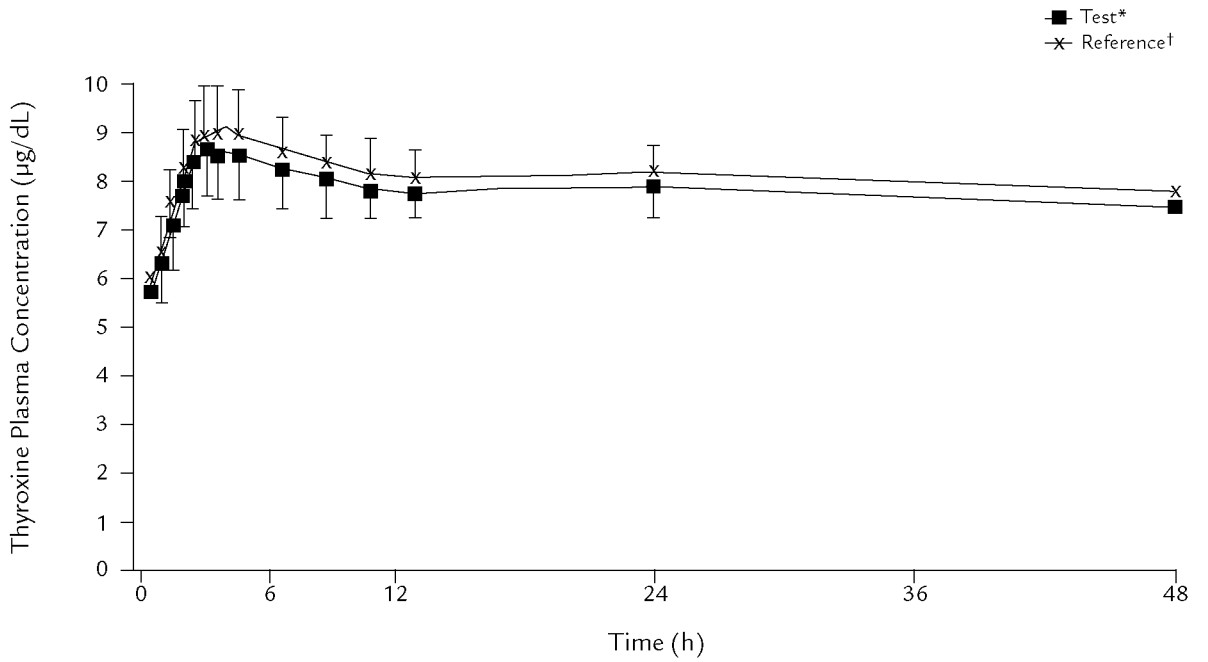

Figure 1. Mean (SD) thyroxine concentration–time profiles after single-dose administration of a test and reference formulation of levothyroxine sodium 600 µg, uncorrected for endogenous thyroxine baseline concentrations. *Trademark: T4 Montpellier® 100 (Química Montpellier S.A., Buenos Aires, Argentina); †Trademark: Synthroid® (Abbott Laboratories, Abbott Park, Illinois).

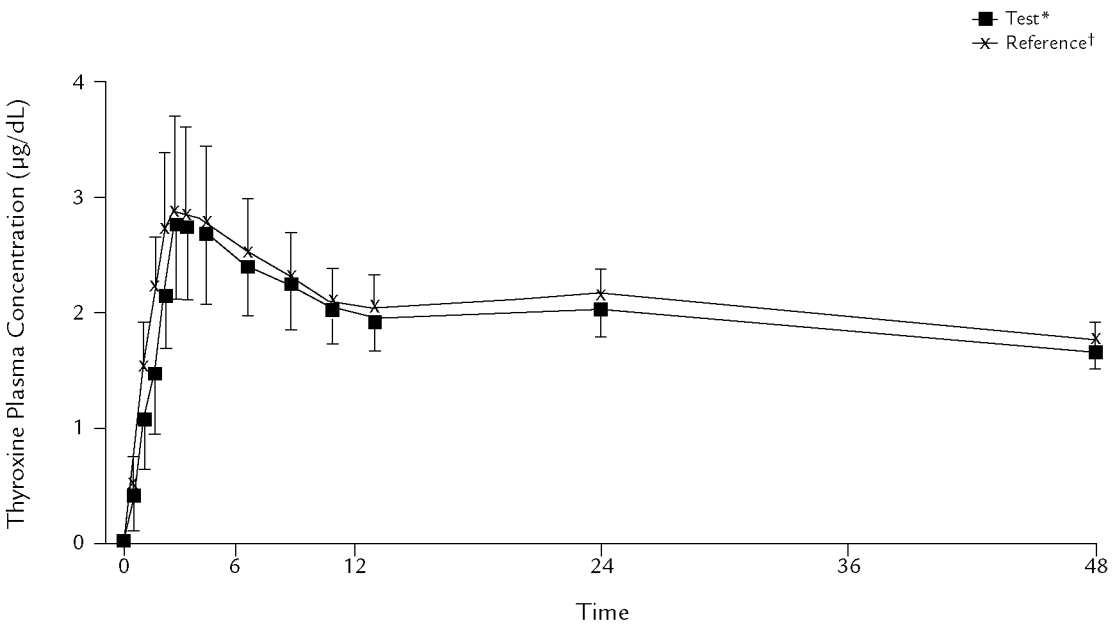

Figure 2. Mean (SD) thyroxine concentration–time profiles after single-dose administration of a test and reference formulation of levothyroxine sodium 600 µg, corrected for endogenous thyroxine baseline concentrations. *Trademark: T4 Montpellier® 100 (Química Montpellier S.A., Buenos Aires, Argentina); †Trademark: Synthroid® (Abbott Laboratories, Abbott Park, Illinois).

Table I. Pharmacokinetic parameters of 2 formulations of levothyroxine without and with correcting for endogenous thyroxine baseline concentrations.

Pharmacokinetic Parameter	Test Formulation*		Reference Formulation†	
	Without Correcting	With Correcting	Without Correcting	With Correcting
T_{max}, h				
Median	2.75	–	2.50	–
Geometric mean	2.92	–	2.74	–
90% CI	2.52–3.98	–	2.50–3.46	–
Arithmetic mean (SD)	3.25 (2.08)	–	2.98 (1.36)	–
CV (%)	64.0	–	45.8	–
Range	1.50–2.75	–	1.50–2.50	–
C_{max}, µg/mL				
Geometric mean	8.92	3.16	9.39	3.39
90% CI	8.53–9.49	2.93–3.68	8.97–10.00	3.15–3.91
Arithmetic mean (SD)	9.01 (1.38)	3.31 (1.06)	9.48 (1.48)	3.53 (1.08)
CV (%)	15.2	32.8	15.6	32.1
AUC_{0-48h}, µg/mL · h^{-1}				
Geometric mean	368.40	88.33	383.37	95.60
90% CI	352.90–391.40	83.56–112.39	368.62–404.00	89.26–113.19

*Trademark: T4 Montpellier® 100 (Química Montpellier S.A., Buenos Aires, Argentina).
†Trademark: Synthroid® (Abbott Laboratories, Abbott Park, Illinois).

ues of C_{max} and AUC_{0-48h}, and median and range for T_{max}. No significant difference in C_{max} and AUC_{0-48h} were observed between both formulations. No subject, treatment, or period effects were observed for the pharmacokinetic parameters studied (data not shown).

Regarding the TSH levels, no significant differences between test and reference were detected at 30 minutes before administration (1.76 [1.05] and 1.72 [0.89] mUI/mL, respectively) or 48 hours after administration (0.92 [0.42] and 0.74 [0.16] mUI/mL).

Significant reductions in TSH values were detected for both formulations at 48 hours after study drug administration compared with values from 30 minutes before administration (both, $P < 0.001$). However, for both formulations, TSH levels were within the normal range (0.4–4.2 mUI/mL). Table II summarizes the bioequivalence analysis of individual C_{max} and AUC_{0-48h} of levothyroxine without and with correction for endogenous levothyroxine baseline concentrations. ANOVA of log-transformed data did not suggest differences between formulations for C_{max} and AUC_{0-48h}. No period effect was detected. Nonparametric analysis (Schuirmann[16] and Anderson and Hauck[17] tests) found that the 90% CI of the test:reference geometric means ratios for C_{max} and AUC_{0-48h} were within the accepted limits of 0.80 to 1.25, indicating that the formulations were bioequivalent, based on the FDA regulatory definition of bioequivalence. Bioequivalence between formulations was found without or with adjustment for baseline hormone levels.

Mean C_{max} and AUC_{0-48h} before and after adjustment for weight were not significantly different; discrepancy between means was <2%, by MANCOVA. We did not find any significant differences, with regard to sex, in the main pharmacokinetic parameters (data not shown).

No adverse events were observed or reported.

DISCUSSION

The results of this bioequivalence study of levothyroxine in normal volunteers suggest the equivalence of the test and reference formulations. In addition, it provided the opportunity to assess the effect of baseline correction on levothyroxine measurements.

The sample studied included 24 volunteers in accordance with current FDA guidelines[8] and similar to recent bioequivalence studies of levothyroxine.[19,20] It was sufficient to meet the regulatory definition of bioequivalence, with and without adjustment for baseline hormone levels, as recommended by Blakesley.[9,21] A washout period of 65 days resulted in no carryover effect.

The issue of correcting actual values according to baseline levels is controversial and has been the subject of intense discussion[21–24] because levothyroxine is naturally present in the blood at levels ranging from 5 to 12 μg/dL. Results from several bioavailability studies and a stochastic simulation of levothyroxine products have suggested that the use of baseline uncorrected C_{max} and AUC_{0-48h} values resulted in 2 products being declared bioequivalent when they actually differed by as much as 35%.[9,10,22,25]

Studies conducted in accordance with FDA guidance aim to establish bioequivalence for products that

Table II. Bioequivalence analysis without and with correcting for endogenous thyroxine baseline concentrations for C_{max} and AUC_{0-48h} mean test*:reference† ratios and CIs.

	Statistical Analysis			
	Without Correcting		After Correcting	
Parameter	Geometric Mean	90% CI	Geometric Mean	90% CI
C_{max}, % ratio	95.06	91.93–98.30	93.13	84.90–102.17
AUC_{0-48h}, % ratio	96.10	94.03–98.21	92.39	85.20–100.20

*Trademark: T4 Montpellier® 100 (Química Montpellier S.A., Buenos Aires, Argentina).
†Trademark: Synthroid® (Abbott Laboratories, Abbott Park, Illinois).

differ by <20% and differentiate and reject as nonequivalent those products that differ by >20%. The question of whether this range is acceptable for levothyroxine, a drug with an NTR, is still unanswered.[23]

Because levothyroxine has an NTR, its well tolerated and effective use requires careful titration and close clinical follow-up. If treatment is not carefully monitored, a patient might be at risk for iatrogenic hyperthyroidism or hypothyroidism.[3,4] Even in the presence of mild (subclinical) hypothyroidism, there is a potential for cardiovascular adverse events such as hypercholesterolemia,[26] increased fibrinolytic activity,[27] systolic and diastolic dysfunction,[28,29] atherosclerosis,[30] arrhythmias,[31] and myocardial infarction.[32]

Guidelines from endocrinology societies (American Association of Clinical Endocrinologists,[33] Endocrine Society,[22,34] and American Thyroid Association[35–37]) advise careful titration of the dose with monitoring and retitration should the dose or brand of drug change, and emphasize the assessment of the bioequivalence to ensure generics also provide therapeutic equivalence for their patients.[10]

When the baseline endogenous level is a significant part of the total drug measured, as was the case in our study, bioequivalence evaluation might be compromised. The relatively high endogenous level of drug results in a masking of the relative difference between the drug products.[23] Compared with our data, patients in the Blakesley et al[9] study presented higher baseline values (within the normal range). Alternatively, bioequivalence trials might be performed in athyreotic subjects, as recently suggested,[38] avoiding the confounding effects of endogenous levothyroxine; however, this approach is very difficult in the clinical setting and includes people that do not qualify as healthy subjects.

Because T_4, triiodothyronine, and TSH levels present diurnal and temporal fluctuations, the FDA[8] has argued against recommending baseline correction in current guidelines. Our results support the suggestions of Blakesley[9,21] that such correction provides more accurate results than current guidelines.

Another proposed recommendation is to use TSH as a surrogate marker, instead of levothyroxine.[9,23] This recommendation was based on the fact that TSH is used clinically to adjust levothyroxine dosage and has been extensively discussed elsewhere.[9,23] Since 2005, the possible use of TSH as a marker has been dismissed by the FDA.[39] In our patients, TSH values were used as a secondary marker of the presence of bioactive levothyroxine, and the reduction in TSH values was similar for both products.

We suggest that an approach involving correction provides appropriate results. However, even after correction based on levothyroxine baseline levels, or the use of longer washout periods, ideal designs to determine bioequivalence of levothyroxine formulations are still a matter for scientific discussion and future research.

Limitations

Bioequivalence studies are typically open label, even when drug concentration readings are blinded. The assumptions are made on a single-dose administration to healthy volunteers (as recommended by FDA, ANMAT, and other regulatory agencies), and the generalization to diseased subjects is, therefore, limited by the design.

CONCLUSION

The results of this study suggest that the test formulation was bioequivalent to the reference formulation of levothyroxine in these healthy volunteers, according to the US FDA definition of bioequivalence. This was supported by the analysis of concentration–time profiles without and with correction for basal endogenous levothyroxine.

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