Current Methodology to Assess Bioequivalence of Levothyroxine Sodium Products Is Inadequate

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ABSTRACT

Levothyroxine sodium is a drug with a narrow therapeutic index for which an individual patient must have his or her dose carefully titrated to achieve the necessary therapeutic effect. In addition, exogenous levothyroxine cannot be distinguished from the endogenously produced hormone. Since 2004, generic formulations have been approved for the most frequently prescribed brands of levothyroxine sodium. This review examines the methodology and statistical acceptance criteria and summarizes findings of a previously published relative bioavailability study that brings into question the use of standard criteria to assess bioequivalence of levothyroxine sodium. The key findings reviewed were the following: (1) in the absence of baseline correction for endogenous T4 levels, products that differed by as much as 25% to 33% would be declared bioequivalent; (2) the use of baseline correction reduced the likelihood of declaring products bioequivalent when they actually differed by 25% to 33%; (3) even with baseline correction, products that differed by 12.5% would be declared bioequivalent; and (4) there was evidence of significant carryover from one dosing period to the next even with washout periods of up to 53 days. In conclusion, the current recommended methodology in the United States to assess bioequivalence for levothyroxine sodium products is inadequate to differentiate products that differ by 12.5%, a clinically relevant difference. Recommendations are made for modifications to the criteria that could improve the likelihood that products that differ by a clinically significant amount in their bioavailability would not be accepted as bioequivalent.

INTRODUCTION

The ultimate objective for defining and then using a methodology to assess bioequivalence of various drug formulations is to provide alternative safe and effective drugs to patients. The key requirement is that pharmaceutically equivalent drugs assessed as bioequivalent, and then approved as therapeutically equivalent, will provide the same clinical effect in patients.

This article provides scientific arguments and data that suggest that the current regulatory methodology to assess bioequivalence in the United States is not sensitive enough to detect potentially clinically significant differences in the bioavailability of levothyroxine products.

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OVERVIEW OF THYROID HORMONES AND LEVOTHYROXINE

Levothyroxine sodium is the sodium salt of the levo isomer of the thyroid hormone thyroxine. Levothyroxine sodium is a drug with a narrow therapeutic index (NTI), defined as a drug that is subject to therapeutic drug concentration or pharmacodynamic monitoring, and/or where product labeling indicates an NTI designation.[1] To understand the underlying physiologic milieu that dictates this NTI, it is essential to understand the control of the production of endogenous thyroid hormone.

Thyroxine (T_4) is the endogenous hormone, synthesized in and released from the thyroid gland. It is a "pro-hormone" in that it is converted in the body to the short-lived, more biologically potent triiodothyronine (T_3). Both T_4 and T_3 affect protein, lipid, and carbohydrate metabolism, growth, and development. They stimulate the oxygen consumption of most cells of the body, resulting in increased energy expenditure and heat production. T_4 and T_3 also possess a cardiac stimulatory effect that may result from direct action on the heart.

The thyroid hormone system is highly regulated through a tight feedback system via the hypothalamic-pituitary-thyroid gland axis. When thyroid hormone levels are low, the hypothalamus secretes thyroid stimulating hormone-releasing hormone (TRH), which stimulates the pituitary gland to produce thyroid-stimulating hormone (TSH). TSH, in turn, stimulates the thyroid gland to produce both T_4 (the major component) and some T_3. When thyroid hormone levels are high, the synthesis and release of TRH and TSH are inhibited, thus resulting in decreased thyroid hormone production and release from the thyroid gland.[2]

Orally administered synthetic levothyroxine is approved for use in the treatment of hypothyroidism, as a TSH suppressant for various types of goiters, and as an adjunct to surgery and radioiodine therapy in the management of thyroid cancer.[3,4] Levothyroxine is prescribed for patients as young as newborns

and is widely used by geriatric patients, patients with underlying coronary heart disease, and in pregnant and nursing women.

As outlined by the Food and Drug Administration (FDA), levothyroxine must be precisely and consistently dosed for it to be safe and effective. "If a drug product of lesser potency or bioavailability is substituted in the regimen of a patient who has been controlled on one product, a suboptimal response and hypothyroidism could result. Conversely, substitution of a drug product of greater potency or bioavailability could result in toxic manifestations of hyperthyroidism such a cardiac pain, palpitations, or cardiac arrhythmias. In patients with coronary heart disease, even a small increase in the dose of levothyroxine may be hazardous."[1] Multiple dosage strengths (ie, 25, 50, 75, 88, 100, 112, 125, 137, 150, 175, 200, and 300 μg) are available for precise titration to meet an individual patient's specific needs. In the range of 75 to 150 μg, the adjacent dosage strengths differ by 9% to 14%, a difference that is used by physicians to carefully titrate an individual patient's dose to achieve the optimal therapeutic effect.

The plasma TSH level is the accepted marker of the thyroid function. Careful dose titration to a target TSH level is the clinical standard-of-care. The relationship between plasma T_4 levels and TSH is an inverse linear:log relationship, wherein a 2-fold change in free T_4 levels is associated with a 100-fold change in TSH levels.[5] Labeling for levothyroxine products recommends dosing adjustments of 12.5 to 25 μg for elderly patients with underlying cardiac disease and patients with severe hypothyroidism until the patient with primary hypothyroidism is clinically euthyroid and the serum TSH has normalized. Both the American Thyroid Association and the American Association of Clinical Endocrinologists have endorsed guidelines that stress careful titration and maintenance of dosing to achieve the optimal clinical outcome.[6]

It is imperative to the health of ~13 million thyroid patients in the United States that levothyroxine sodium products perform reliably and predictably. Prior to June 2004, the only approved levothyroxine products were BX-rated and thus nonsubstitutable one for the other. In this circumstance, the patient is titrated to a consistently uniform levothyroxine preparation based on clinical manifestations and then kept on that same preparation chronically. With any change in preparation, the patient would be monitored and again titrated, if necessary, to optimal clinical response. Since June 2004, generic levothyroxine products have been approved and designated as AB-rated (substitutable) to the branded products. In this circumstance, different products are deemed therapeutically equivalent and may be switched among any of the AB-rated products from prescription refill to prescription refill.

Thyroid experts note that levothyroxine products that provided differences of 10% or greater would not provide a similar therapeutic response, particularly in subsets of patients for whom tight control was particularly necessary (ie, elderly patients with cardiac disease, patients with thyroid cancer, and newborns and young children). Thus, it is essential to patient health that the generic products are in fact therapeutically equivalent to the brand for which they can be substituted.

BIOEQUIVALENCE QUESTION

The assessment and approval of a generic product as AB-rated to the branded levothyroxine product is dependent on demonstrating both pharmaceutical equivalence and bioequivalence. Bioavailability is assessed by administering supraphysiologic doses of levothyroxine to healthy normal volunteers who produce endogenous levothyroxine. The T_4 bioavailability of the generic product relative to that of the reference branded product is assessed by the 2 one-sided tests procedure via 90% confidence intervals obtained from the analysis of the natural logarithms of area under the curve (AUC) and C_{max}. Bioequivalence is concluded if the 90% confidence intervals for the relative bioavailability are within the 0.80 to 1.25 range.

In the case of levothyroxine products, these standard pharmacokinetic (PK) measurements and statistical criteria to assess product bioequivalence, however, would not be adequate to ensure therapeutic equivalence. This concern is based on an understanding of thyroid hormone physiology and the need for careful tight dose titration, as well as observations made as a result of the bioavailability studies for Synthroid. The standard regulatory methodology for bioequivalence was originally designed for exogenous compounds, and a thorough assessment is required before such a methodology is applied to more complex drugs such as an endogenous hormone, as is the case for levothyroxine. Some of the challenges unique to assessing the bioavailability of levothyroxine and methods to address some of these problems were considered prior to the issuance of the guidance for assessment of relative bioavailability of levothyroxine sodium products.[1] First, exogenous levothyroxine is biochemically and physiologically indistinguishable from endogenously produced T_4, precluding an easy method of distinguishing the exogenous and endogenous sources of the measured T_4 levels in the blood. In healthy volunteers, the endogenous total T_4 levels range from 5.0 to 12.0 μg/dL. The exogenous levothyroxine dose must be sufficiently large to detect the contribution of that dose above and beyond the endogenous T_4 level. It was recognized that the exogenous levothyroxine dose needed to be several fold over the normal treatment dose to raise the levels of T_4 significantly above baseline levels to allow measurement of the exogenous T_4. Second, T_4 has a long half-life of 6 to 9 days, making it necessary to incorporate a long washout period between dosing periods.

Given the potential problem of applying the standard regulatory methodology designed to assess bioequivalence for

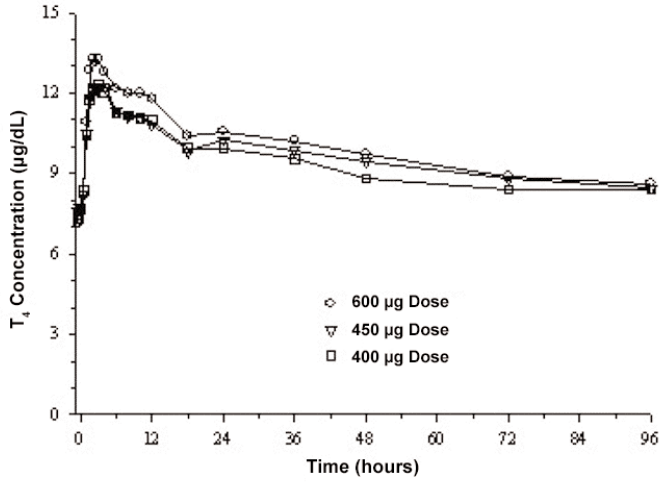

Figure 1. Mean levothyroxine (T_4) concentration-time profiles on study day 1 following single dose administration of levothyroxine sodium – uncorrected for endogenous T_4 baseline concentrations.

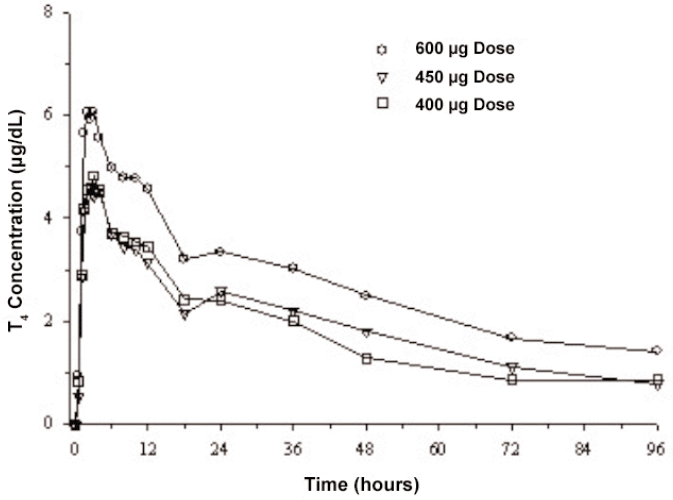

Figure 2. Mean levothyroxine (T_4) concentration-time profiles after correction for endogenous baseline levels of T_4 using Correction Method 1.

exogenous compounds to levothyroxine, the logical question to specifically ask, therefore, was whether the standard method used to assess bioequivalence was sensitive enough to discriminate 2 products that differed in bioavailability by a clinically significant amount.

Results of a Relative Bioavailability Study of Levothyroxine

A relative bioavailability study was undertaken to test the sensitivity of the recommended methodology by the FDA to assess bioequivalence of levothyroxine sodium products.[7] The objective of the study was to test the sensitivity of the recommended methodology to distinguish between dosing regimens of levothyroxine sodium known to differ in the amount of drug available.

The design was a 3-way crossover study in healthy subjects with a single dose given in each dosing period. All tablets (50 µg strength) used throughout the study were from the same lot of an approved levothyroxine sodium product (ie, Synthroid). Three different doses were administered; each dose could represent a theoretical product. Because the same formulation was used for the 3 dosing periods, any differences in drug availability would not be owing to differences in dissolution and absorption. Supraphysiologic doses were used to allow measurement of T_4 as an incremental increase over the endogenous plasma T_4 levels. The doses were several-fold over normal clinical doses, as recommended by the FDA in the guidance issued for the studies required for new drug application (NDA) submission of levothyroxine sodium products.[1]

Three different methods of baseline correction to adjust for the amount of endogenous T_4 in healthy euthyroid volunteers were evaluated. Baseline correction methods for endogenous T_4 levels were done using each of the following 3 methods:

- Correction Method 1. For each subject and period the mean of the 3 T_4 values at –0.5, –0.25, and 0 hours before dosing was subtracted from each T_4 concentration after dosing.
- Correction Method 2. For each subject and period, each T_4 concentration after dosing was corrected for the hypothetical decay of endogenous T_4 with a 7-day half-life, beginning with the level obtained immediately after dosing.
- Correction Method 3. For each subject and period, each T_4 concentration was measured at the analogous time point on the day prior to administration of the levothyroxine dose of each period.

Standard PK measurements describing the rate and extent of T_4 absorption (eg, C_{max} and AUC) were obtained. The mean T_4 serum concentration-time profiles after each of the 400-, 450-, and 600-µg doses of levothyroxine sodium, without any correction for baseline T_4 levels, are presented in Figure 1.

The mean T_4 levels before dosing were in the 7 to 8 µg/dL range for each dose (well within the range known for healthy volunteers) and reached 13 to 14 µg/dL after drug administration, before declining.

As an example of the effect of adjusting for endogenous T_4 levels, the mean T_4 concentration-time profiles after each of the 400-, 450-, and 600-µg doses of levothyroxine sodium using correction Method 1 are shown in Figure 2. The mean serum T_4 concentrations after correction of baseline T_4 levels were higher after administration of the 600-µg dose than after the 400- and 450-µg doses.

Table 1. Bioequivalence and Relative Bioavailability for Levothyroxine (Correction Method 1)*

Regimens				**Relative Bioavailability**	
Test vs	Pharmacokinetic	Central Value†		Point	90% Confidence
Reference	Parameter C_{max}, μg/dL AUC, μg · hr/dL	Test	Reference	Estimate‡	Interval
450 μg vs 600 μg	Cmax	5.4	6.9	0.783	0.727 - 0.844
		119.7	167.3	0.715	0.658 - 0.778
400 μg vs 600 μg	Cmax	5.6	6.9	0.803	0.745 - 0.865
		118.9	167.3	0.711	0.653 - 0.773
450 μg vs 400 μg	Cmax	5.4	5.6	0.975	0.906 - 1.049
		119.7	118.9	1.007	0.926 - 1.094

*Adapted from Blakesley et al.[7]

†Antilogarithm of the least squares means for logarithms.

‡Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

If 2 products have identical bioavailabilities, the point estimate from a study of relative bioavailability should be near 1.00. Theoretically the point estimates of the 400- and 450-μg doses relative to the 600-μg dose should be 0.67 and 0.75, respectively. Without baseline correction, the point estimates for AUC_{48} were 0.930 and 0.954 for the 400- and 450-μg doses relative to the 600-μg dose, respectively. Using Method 1 to correct baseline T_4 levels, the point estimates for the 400- and 450-μg doses compared with the 600-μg dose were 0.711 and 0.715, respectively (see Table 1). The closer correlations of the measured point estimates to the actual relative bioavailabilities of doses of the same formulation of levothyroxine indicate the usefulness of applying a correction method to adjust for the baseline (endogenous) T_4 levels.

Using standard statistical criteria, bioequivalence was concluded if the 90% confidence intervals from the analyses of the natural logarithms of AUC and C_{max} were within the 0.80 to 1.25 range.[8] An example of the effect of baseline correction on the relative bioavailabilities of the 3 doses using Method 1 to correct for baseline T_4 levels is presented in Table 1.[7]

The key results of the relative bioavailability study[7] are summarized as follows:

- First, the use of baseline uncorrected T_4 C_{max} and AUC_{48} values would result in declaring 2 products bioequivalent when they actually differ by as much as 25% to 33% (450 μg and 400 μg vs 600 μg).
- Second, the use of baseline corrected C_{max} and AUC_{48} values would reduce the likelihood that 2 products would be declared bioequivalent when they actually differ by 25% to 33%.
- Third, the 450-μg dose would continue to be declared bioequivalent to the 400-μg dose using the C_{max} and AUC_{48} values for the uncorrected T_4 data or the baseline-corrected T_4 data by any of the 3 methods.
- Fourth, there was evidence of significant carryover from one dosing period to the next even with washout periods of up to 53 days.

DISCUSSION

The results of the relative bioavailability of 3 known doses of levothyroxine substantiate the concerns of applying the standard methodology and statistical criteria to assess bioequivalence of levothyroxine. Of particular significance, the 450-μg dose differs by 12.5% from the 400-μg dose but would be declared as bioequivalent when using the standard methodology and statistical criteria, even with baseline correction. The report also describes results of 2 other methods to correct for the baseline T_4 level. Although there were some quantitative improvements in the calculated estimates of the relative bioavailabilities of the 3 doses (ie, point estimates were closer to the actual ratio of doses and 90% confidence intervals contained the actual difference ratio), the pattern for determination of bioequivalence using the standard statistical criteria was the same as for the correction Method 1.

The finding of a carryover effect after a washout period of 5 to 8 times longer than the T_4 half-life also indicates a unique challenge of applying standard methodology to assess bioequivalence of levothyroxine products.

The authors concluded that the application of criteria for determination of bioequivalence without accounting for endogenous T_4 levels resulted in failure to identify products that differed by as much 25% to 33% and that products that differ by 12.5% could be declared as bioequivalent even with baseline correction.[7]

Based on the results of this study, the FDA adopted the use of baseline correction with Method 1 in assessing relative bioavailability. The standard statistical criteria of the 90%

confidence interval within an acceptance range of 0.8 to 1.25 remained the same.

Further examination of the results achieved using Method 1 to correct for baseline T_4 levels shows that the AUC_{48} point estimate of the 450-µg dose compared with the 400-µg dose is 1.007, when the actual value is 1.125. In addition, the 90% confidence interval for AUC_{48} includes unity (ie, 1.000) but does not include the actual value of 1.125. In fact, this relatively narrow 90% confidence interval is easily contained within the 0.8 to 1.25 range. The width of the 90% confidence interval affects the determination of bioequivalence. The narrower the confidence interval, the further the point estimate may drift from 1.000 and still result in bioequivalence. Based in part on results of this study, it would not be surprising that a levothyroxine product that differs by 12.5% or more, up to 25%, from the reference levothyroxine product could pass as bioequivalent.

CONCLUSION

Levothyroxine sodium is a drug with an NTI, of which physicians are fully cognizant. Current levothyroxine product labels and guidelines from leading endocrinology societies advise careful titration of the dose with monitoring and retitration should the dose or brand of drug change. Now that generic drugs have been approved as fully substitutable with the prescribed products, physicians rely on the assessment of bioequivalence to ensure generics also provide therapeutic equivalence for their patients. The current methodology to assess bioequivalence and assign therapeutic equivalence is inadequate to meet the clinical needs of thyroid patients. Therapeutically equivalent products, according to the FDA, "can be substituted with the full expectation that the substituted product will produce the same clinical effect and safety profile as the prescribed product."[9]

The final outcome is that current methodology to assess bioequivalence allows levothyroxine sodium products that differ by more than 12.5% (a clinically significant amount) to be declared therapeutically equivalent. Therefore, even including recently instituted baseline correction, the current methodology to assess bioequivalence and assign therapeutic equivalence is inadequate to meet the clinical needs of thyroid patients.

The methodology used to demonstrate bioequivalence is critical. It must be sensitive enough to detect a "significant difference" between the test (generic) and the reference (branded) products. A methodology that cannot detect a significant difference in the rate and extent of absorption between the test and reference products fails to provide assurance that substituted levothyroxine sodium products will provide equivalent therapeutic benefit to thyroid patients.

At a minimum, a bioequivalence study must include 2 elements. First, it must include appropriate measures by which to compare the release and absorption of levothyroxine from each product. Second, the statistical acceptance criteria must ensure that the risk is small that levothyroxine products with bioavailabilities that differ by a clinically significant amount will pass as bioequivalent.

This author recommends that the methodology used to assess bioequivalence of levothyroxine sodium products be thoroughly reevaluated in light of the unique attributes of thyroid hormone physiology, and that necessary modifications in the methodology, such as narrowing the acceptance range, be implemented to ensure that levothyroxine sodium products deemed therapeutically equivalent will indeed produce the same clinical effect and safety profile as the prescribed product.

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