In Vitro Dissolution and In Vivo Bioavailability of Commercial Levothyroxine Sodium Tablets in the Hypothyroid Dog Model

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Abstract □ The objective of this study was to determine whether a correlation exists between the rate of in vitro dissolution and bioavailability of levothyroxine sodium (T_4) tablets. Dissolution versus time profiles for Synthroid, the Flint brand of levothyroxine sodium, and two competitors' tablets (brands A and B) were generated using an official dissolution apparatus (USP), and 0.05 M phosphate buffer (pH 7.4) as the medium. These tablets were also utilized in single-dose crossover bioavailability studies in the hypothyroid dog model (n = 6). The average areas under the serum T_4 concentration versus time curve from 0 to 8 h (AUC) for Synthroid, brand A, and brand B were 8.22, 6.32, and 8.70 ng-h/mL per dose (μg per kg body weight), respectively. Respective peak serum concentrations (C_{max}) for each tablet formulation were 1.26, 1.07, and 1.36 ng/mL per dose. The corresponding dissolution rates, expressed as $t_{50\%}$, were 20.5, 3.06, and 14.1 min, respectively. Data analysis indicated no correlation between dissolution kinetic parameters and the bioavailability parameters AUC and C_{max}. However, a linear relationship was observed between dissolution kinetics and both the time to reach maximal serum concentration (t_{max}) and the observed absorption rate constant (k_a).

Levothyroxine sodium is utilized for the treatment of hypothyroidism and suppression of the thyroid–pituitary axis (e.g., goiter suppression or treatment of thyroidal cancer). Despite the widespread use of both generic and brand name products of levothyroxine, there are few data available comparing bioavailability of different tablet preparations.[1–11] The majority of these published studies were not designed to determine the influence of formulation factors on the absorption of levothyroxine. Moreover, in several studies, the drug content in tablets was not determined during the course of the study. As a result, the bioavailability differences observed between formulations cannot be attributed to differences in formulation factors. In fact, a number of reports in the literature describe large variations between actual and labeled levothyroxine contents of commercial tablets.[4–8]

An in vitro dissolution test can be a sensitive method for differentiating between formulations of the same therapeutic agent. First, it can be utilized as a quality control tool to ensure manufacturing reproducibility from lot to lot. Secondly, if a correlation between in vitro dissolution and bioavailability is established, it may be indicative of the effect of formulation factors on the bioavailability of drugs.

The present study was undertaken to determine and compare dissolution rates of levothyroxine from three commercially available products in phosphate buffer using the USP apparatus, to determine and compare bioavailability of levothyroxine from these products, and finally to investigate correlations of in vivo parameters with in vitro dissolution parameters.

Experimental Section

A single lot of levothyroxine sodium tablets (200 μg) from each of three brands was utilized in the bioavailability and dissolution studies. One of the brands used was that of the innovator, Synthroid from Boots-Flint (Lincolnshire, IL). The other two brands used in these studies will be identified as brands A and B.

Bioavailability Studies—Six male beagle dogs (Laboratory Research Enterprises, Kalamazoo, MI), with initial weights ranging between 10 and 15 kg, were made hypothyroid by intravenous injection of 5–13 mCi of NaI^{131} (New England Nuclear, Boston, MA). At least 5 weeks were allowed between the treatment with radioactive iodide and the initiation of the bioavailability studies. During that time, the serum thyroxine concentration in these dogs declined ~25% of the pretreatment value, and serum radioactivity returned to the background level.

Balanced crossover designs were employed to compare the bioavailability of levothyroxine sodium tablets from each of the three brands. Levothyroxine was administered orally as weighed half-tablets of the assigned brand. The remaining half-tablets were weighed and analyzed for levothyroxine content by high-performance liquid chromatography (HPLC) as described under *Dissolution Studies*. The nominal levothyroxine sodium content per tablet of all brands is shown in Table I. Blood samples were taken from each dog in each treatment period just before receiving the drug (time 0) and subsequently at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 24 h. Blood samples were centrifuged immediately and the serum was frozen for the analysis of levothyroxine by a radioimmune assay using the Gammacoat test kit[12] (Clinical Assays, Boston, MA). The Gammacoat kit method quantitatively measures levothyroxine using a standard curve generated from levothyroxine standards. It is a rapid, precise, accurate, and simple assay to perform. The limit of detection for levothyroxine is 0.013 ng/mL. At least 48 h were allowed between treatments; during this period, serum levothyroxine concentration returned to pretreatment values.

Dissolution Studies—Dissolution experiments were performed using the dissolution apparatus II (Hanson Research Corporation, Northridge, CA), paddle method (USP XXI). The dissolution rate from a test tablet was determined in 500 mL of phosphate buffer (pH 7.4) with a paddle speed of 100 rpm. Following introduction of a test tablet into a dissolution flask, 50-mL aliquots were withdrawn and 50 mL of fresh phosphate buffer (pH 7.4 and 37 °C) was added back to the flask at predetermined sampling intervals. Each sample was filtered through a 0.8-μm polycarbonate membrane (Nucleopore, Pleasanton, CA), discarding the first 10 mL. A 20-mL aliquot of each sample was then taken, to which was added 1.0 mL of 2% ammonium hydroxide in methanol solution.

Levothyroxine concentration was determined using a modification of a previously reported HPLC method.[13] In brief, a reversed-phase C-18 μBondapak column (Millipore, Milford, MA) was used, preceded

Table I—Levothyroxine Sodium Content of Various 200-mg Tablets Used in the Bioavailability Studies

Tablet Brand	n	Levothyroxine Sodium Content, μg/tablet (Mean ± SD)[a]	95% Confidence Interval
Synthroid	20	200.2 ± 8.5	196.2–204.2
Brand A	20	195.0 ± 8.7	190.0–199.1
Brand B	20	172.3 ± 14.1	165.7–178.9

[a] Determined before the expiration date; based on half-tablet determinations.

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by a C-18 pre-column (Millipore, Milford, MA). The mobile phase consisted of methanol:0.085% phosphoric acid (60:40) at a flow rate of 2.0 mL/min, using an M-45 pump (Millipore, Milford, MA). Levothyroxine was detected by UV spectroscopy (Spectroflow 783, Kratos Analytical Instruments, Ramsey, NJ) at 225 nm. Sample injection volumes of 200 μL were used.

Concentrations of samples were determined by linear regression of standard chromatographic peak heights. Standards were prepared using levothyroxine USP reference standard and were treated identically to samples except they contained no tablet excipients. This method is precise over the concentration range 10–400 ng/mL. The within run variability was 3–5%, and the between run variability was ~4–7%. Regression coefficients were 0.99 or better.

Determination of Tablet Potency—The analytical method used to determine levothyroxine content of representative tablets used in these studies is described in detail elsewhere.[14] In brief, a known quantity of tablets from each manufacturer's lot was placed in a 250-mL glass-stoppered Erlenmeyer flask containing 100 mL of methanolic NaOH. The flasks were then shaken for 35–45 min which was ample time to allow complete disintegration of the tablets. An 8–10-mL aliquot was then removed, filtered, and diluted, depending on the dosage of the tablet. The sample was then assayed by HPLC. The HPLC system was similar to that described in the dissolution studies. The mobile phase consisted of 60% methanol and 40% of a 0.085% (v/v) solution of phosphoric acid. An external standard curve based on levothyroxine USP reference standard was utilized to quantitate levothyroxine. Injection volumes were 100 μL. This method is precise and specific for levothyroxine.

Results and Discussion

The actual and labeled levothyroxine contents of the actual lots of brands of tablets utilized in both the bioavailability and dissolution studies are shown in Table I. All subsequent dissolution determinations are based on the actual levothyroxine content of the tablets tested. In addition, pharmacokinetic parameters have been normalized based on actual dose of levothyroxine administered as opposed to the labeled dose.

In the pharmacokinetic analysis and subsequent correlations of pharmacokinetic and dissolution kinetic parameters, it is assumed that there is no difference in the in vivo dissolution and subsequent bioavailability between half and whole tablets. The underlying rationale for administering a half tablet to the test animal was because of inconsistencies in the literature regarding equivalence of various levothyroxine preparations.[1–11] In fact, over the past decade, a controversy has existed among thyroidologists in regard to the reasons underlying the observed variations in serum thyroxine concentrations in patients taking different preparations of synthetic levothyroxine. For those studies which have indicated inequivalence, possible explanations offered for the observed variation have included differences in tablet content or formulation differences that could influence tablet dissolution and, ultimately, bioavailability. Indeed, there have been reports published over the past few years which indicate that there is a wide variability between levothyroxine products with regard to the actual versus labeled levothyroxine content.[4–8] Moreover, since the actual levothyroxine content in these tablets is probably time dependent, it was felt necessary that the content of tablet being administered to the dog be known. This alleviates potential intertablet variation as a source of error in the determination of pharmacokinetic parameters. Moreover, with a knowledge of the tablet content, any differences in dose-normalized pharmacokinetic parameters can be attributed to formulation effect. Otherwise, it would not be known whether differences in pharmacokinetic parameters were due to formulation differences or merely differences in tablet content. Therefore, the tablets were halved; one half was assayed for levothyroxine content and the other half was administered orally to the test animal. In this manner, the dose being administered was known. The relatively small standard error and narrow 95% confidence interval, as shown in Table I, indicates that the drug is homogeneously distributed within a tablet.

Obviously, prerequisite to this approach is considering the assumption that there is no difference in the in vivo dissolution and consequently the bioavailability between half and whole tablets of levothyroxine sodium. There have been several reports in the literature which have addressed this issue. For example, Hollander and Hoogslag[15] showed that no differences in release could be detected between half and whole tablets of a disopyramide phosphate formulation. Moreover, there were no clinically relevant differences in blood levels of the drug in a randomized crossover study in which six subjects received one whole tablet or one tablet divided into two halves on two different days. Simons et al.[16] have reported a study in which they have shown that areas under the curve, mean absorption time, and fraction of dose recovered in the urine at 24 h were not significantly different for theopylline tablets ingested either whole or halved in seven healthy adults.

There are no studies reported in the literature comparing the bioavailability of whole versus halved levothyroxine sodium tablets. Nevertheless, there is nothing about the formulations of the brands of levothyroxine sodium tablets used in the present studies to suggest that there would be differences. For example, these tablets are not enterically or otherwise coated, nor are they controlled-release tablets. Differences in bioavailability between whole and halved levothyroxine sodium tablets are not expected provided that pharmacokinetic parameters are normalized for dose. Moreover, relative comparisons in the bioavailability of various brands of levothyroxine are certainly valid upon administration of halved tablets.

In light of the inconsistencies in previously published literature, it was felt that the scientific benefits of knowing more precisely the levothyroxine content of the tablet being administered to the test animal outweigh the risk of the beforementioned assumption.

Pharmacokinetic Analysis—The serum concentration–time data were fitted to the one-compartment open model with first-order absorption and elimination (eq 1) using SAS:[17]

$$C = \frac{FD \cdot k_a}{V_d (k_a - k_{el})} (e^{-k_{el}t} - e^{-k_a t}) \quad (1)$$

where C is the serum concentration of levothyroxine at time t, FD is the fraction of administered dose absorbed, k_a is the absorption rate constant, k_{el} is the elimination rate constant, and V_d is the volume of distribution.

Using this model, the maximal concentration in the serum (C_{max}) and the time to reach maximal concentration in the serum (t_{max}) are computed by eqs 2 and 3, respectively:[18]

$$C_{max} = \frac{k_a F D_0}{V(k_a - k_{el})} (e^{-k_{el}t_{max}} - e^{-k_a t_{max}}) \quad (2)$$

$$t_{max} = \frac{1}{k_a - k_{el}} \ln \frac{k_a}{k_{el}} \quad (3)$$

These pharmacokinetic parameters, as well as others obtained for the different brands, are listed in Table II. The parameters C_{max} and the area under the serum concentration versus time profiles (AUC) are normalized by the dose administered to the animals (D/body weight). A typical fit of the data to eq 1 is shown in Figure 1. Although there are less data in the elimination phase relative to the absorptive phase, the estimate of k_{el} reported in Table II is in agreement with literature.[19] An analysis of variance was used to analyze between brand differences on the parameters in Table II.

There was no significant difference in C_{max}, t_{max}, or AUC among the three brands. It appears that reported differences in serum concentration of levothyroxine following oral ad-

Table II—Pharmacokinetic Parameters for Oral Levothyroxine Sodium Products

Parameter	Brand		
	Synthroid	A	B
C_{max}, (ng·kg/mL·μg)	1.261	1.074	1.359
	(0.146)[a]	(0.146)	(0.146)
t_{max}, h	5.26	6.97	5.81
	(0.535)	(0.535)	(0.535)
k_a, h^{-1}	0.485	0.203	0.312
ln k_a	−0.722	−1.595	−1.164
	(0.137)[b]	(0.137)	(0.137)
k_{el}, h^{-1}	0.054	0.107	0.078
ln k_{el}	−2.916	−2.233	−2.549
	(0.176)[b]	(0.176)	(0.176)
AUC $|_0^8$, (ng·h·kg/mL·μg)	8.22	6.32	8.70
	(0.944)	(0.944)	(0.944)
AUC $|_0^{24}$, (ng·h·kg/mL·μg)	21.2	18.1	22.4
	(2.91)	(2.91)	(2.91)

[a] Numbers in parentheses represent pooled standard error of the mean; the pooled standard deviation was used to reflect that each animal was given multiple brands of levothyroxine. [b] The pooled standard error is presented in logarithmic scale.

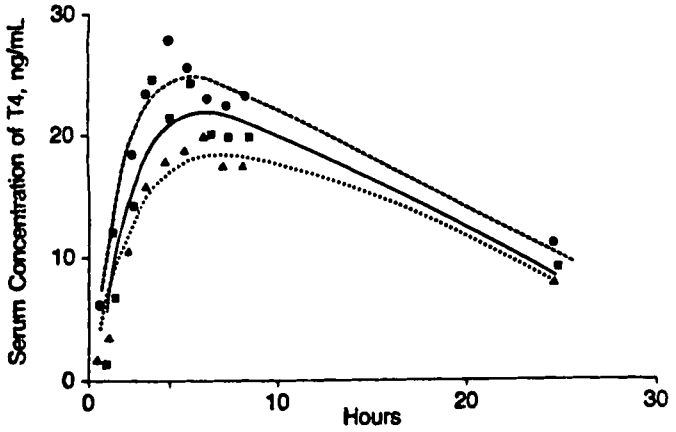

Figure 1—Serum concentration of levothyroxine as a function of time following oral administration of Synthroid, brand A, and brand B tablets (200 μg). Theoretical lines are based on equation (1): (– – – –) Synthroid; (· · · ·) brand A; (———) brand B. Experimentally determined points are: (●) Synthroid; (▲) brand A; (■) brand B.

ministration of tablets from different brands may be attributable, in some instances, to differences between the labeled and actual potency of the tablet.[4–8] The absorption rate constant (k_a) was significantly higher for Synthroid as compared with brands A and B ($p < 0.05$). Nevertheless, k_a alone is not an indication of the bioavailability of a product.

Dissolution Studies—The dissolution data were fitted to a first-order expression similar to those used by Wagner[20] and Gibaldi and Feldman.[21] The expression is:

$$D_t = D_m - Me^{-k_d t} \quad (4)$$

where D_t is the cumulative percent of drug dissolved at any time t during the dissolution test, D_m is the maximum cumulative percent of drug dissolved during the dissolution test, M is a constant, and k_d is an observed first-order dissolution rate constant. Estimates of the parameters D_m, M, and k_d were obtained through the application of nonlinear regression analysis using SAS[11] and are shown in Table III. The suitability of this model is clearly illustrated by Figure 2 which compares the theoretical dissolution profile based on eq 4 with actual dissolution data obtained for the three brands.

Table III—Dissolution Parameters[a] for Levothyroxine Sodium Products Using the USP Apparatus II Method

Product	n	D_{30}, %	D_{60}, %	t_{50}, min	D_M	M	k_d, min^{-1}
Synthroid	6	65.3 (4.8)[b]	80.1 (1.3)	20.5	85.6	111.9	0.056
Brand A	6	91.7 (2.2)	96.5 (2.0)	3.06	96.3	52.6	0.063
Brand B	5	82.0 (1.5)	96.2 (2.0)	14.1	98.7	133.0	0.071

[a] D_{30} is percent dissolved in 30 min, D_{60} is percent dissolved in 60 min, t_{50} is time to reach 50% dissolution, D_M is the maximum cumulative percent of drug dissolved, M is a constant, and k_d is the observed first-order dissolution rate constant. [b] Numbers in parentheses are standard error of the mean.

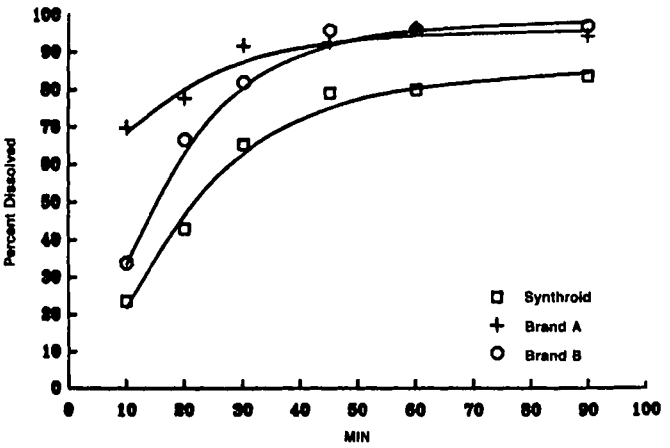

Figure 2—Dissolution profiles for various brands of levothyroxine sodium tablets. Solid line is theoretical based on model (eq 4).

The percent dissolved in 30 min (D_{30}), percent dissolved in 60 min (D_{60}), time to reach 50% dissolution (t_{50}), and the apparent dissolution rate constant (k_d) for each of the brands tested are also found in Table III. A review of these data indicates that of the three brands tested, the Synthroid tablet had the slowest dissolution rate. An analysis of variance was used to compare the parameters in Table III between brands. The experimentally determined parameters D_{30} and D_{60} were significantly lower ($p < 0.05$) for Synthroid compared with either brand A or brand B. In addition, t_{50} was significantly shorter for brand A relative to Synthroid or brand B.

Correlation of Pharmacokinetic and Dissolution Kinetics—Various in vivo and in vitro dissolution parameters were subjected to linear regression analysis and the correlation coefficients (r) were calculated. Those correlated parameters are presented in Table IV. Plots of t_{max} versus D_{30} and k_a versus D_{30} are illustrated in Figures 3 and 4, respectively. These correlations indicate the potential of the experimentally determined parameters D_{30} and D_{60} to predict the pharmacokinetic parameters t_{max} and k_a under the conditions of the dissolution test. Interestingly, it appears that the slower the dissolution kinetics, the faster the pharmacokinetics, as indicated by the positive and negative slopes of

Table IV—In Vivo:In Vitro Correlations[a] for Oral Levothyroxine Brands

Parameter		r
In Vitro	In Vivo	
D_{30}	t_{max}	0.940
D_{30}	k_a	−1.00
D_{60}	k_a	−0.930
t_{50}	k_a	−0.960

[a] The only correlations reported are those having absolute values of $r \geq 0.90$.

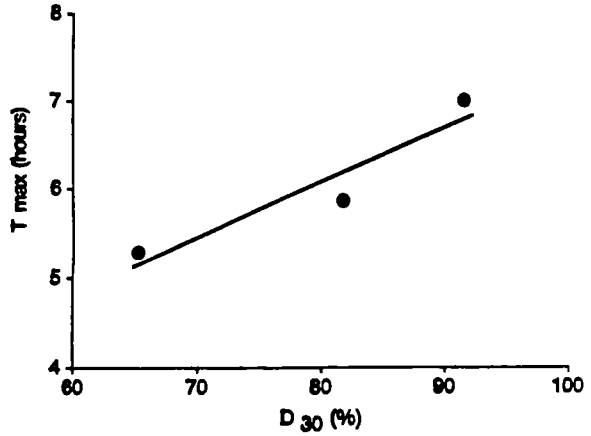

Figure 3—Linear regression plot of time to reach maximal concentration versus percent dissolved in 30 min (D_{30}).

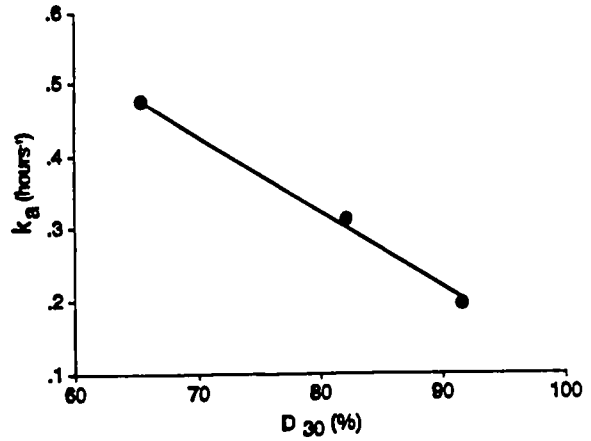

Figure 4—Linear regression plot of absorption rate constant (k_a) versus percent dissolved in 30 min (D_{30}).

Figures 3 and 4, respectively. It would be purely speculative to suggest mechanisms to explain these observed correlations. If the in vivo dissolution rate is rate limiting in the absorption of levothyroxine, then this inverse relationship simply indicates that the conditions of the in vitro dissolution kinetic studies are not representative of the in vivo dissolution kinetic conditions. It could be argued that the dissolution rate of levothyroxine from the Synthroid formulation is actually faster than that from brands A or B in 0.1M HCl which is more representative of the environment of the stomach milieu. However, preliminary experiments indicated that the differences in dissolution rate among the various brands were actually increased under these conditions. It is interesting to note that the selected conditions of the dissolution experiments are exactly those defined in the official USP test for the levothyroxine sodium tablet monograph.[21] Otherwise the inverse relationship implies that the dissolution rate is not the rate-limiting step in drug absorption. It is possible that levothyroxine is not well absorbed from the stomach and that even though t_{50} may be relatively fast for one brand, significant drug is not absorbed until the stomach empties and the drug enters the duodenum. A product such as Synthroid, which has a relatively long t_{50}, may release a significant amount of levothyroxine from its formulation once it has reached the duodenum where it is very quickly absorbed. In fact, Chung and Van Middlesworth[22] have demonstrated, using intestinal segment transport studies, that 50% of a tracer dose of levothyroxine was absorbed in the first hour.

However, the in vivo parameters k_a and t_{max} alone are not indicative of bioavailability. Typically, the area under the serum concentration versus time curve is accepted as a measure by which the bioavailability of a drug from different brands can be compared. A comparison of the in vitro and in vivo parameters shown in Tables II and IV indicates that a correlation between bioavailability (as measured by AUC) and any of the dissolution parameters does not exist. For example, all of the in vitro dissolution parameters obtained for Synthroid suggest the dissolution rate of levothyroxine from this brand is significantly slower than that from the other brands, yet its bioavailability is at least similar if not higher than that of the other brands.

In conclusion, it appears that the dissolution test as described is capable of discriminating various brands of oral levothyroxine in terms of certain pharmacokinetic parameters, namely k_a and t_{max}. However, a correlation between in vitro dissolution and bioavailability as measured by AUC was not apparent under the conditions of the dissolution test. It is quite possible that use of different dissolution media, agitation, etc., could result in correlations between in vitro dissolution and AUC. It should be noted however that the conditions of the dissolution test used in the present studies are those of the official USP test.[23] Due to the potentially complex absorption mechanisms of levothyroxine, development of an in vitro dissolution test which correlates with in vivo bioavailability may be somewhat fortuitous. Nevertheless, the dissolution test under the conditions described, has excellent potential as a quality control tool.

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