INNOVATIVE STUDIES IN WOMEN BY USE OF STABILIZED ISOTOPES IN PREGNANCY

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ABSTRACT

Pharmacokinetic studies are conducted in order to determine drug absorption, distribution, metabolism and excretion. This knowledge serves to help determine the appropriate and timely use of medications and is also an important step in providing individualized therapeutics according to patient characteristics, such as disease state and genotypes of drug metabolizing enzymes. An innovative way of conducting pharmacokinetic research in pregnancy is presented, with the drug levothyroxine (LT4).

The stable, non-radioactive Carbon-13 isotope was used to prepare a derivative of LT4, which was then used to determine the pharmacokinetics of the drug in 9 pregnant women serving as their own controls. Of 9 study subjects, 6 returned to participate in the post partum (non-pregnant) portion of the study. The median time to peak blood level was determined to be at 8 hours post dose. The $AUC_{0-\infty}$ results were significantly higher during pregnancy than in the same woman approximately 6 months post partum. The increase in LT4 AUC during pregnancy could be attributed to a decrease in LT4 clearance. Additionally, a large variability in the pharmacokinetics of LT4 was found in pregnant women, and a relatively narrower range of variability in non-pregnant women. In order to solidify these findings, a larger group of patients is required. In addition, the mechanisms responsible for the gestational differences in pharmacokinetics need to be investigated.

Key Words: *Levothyroxine, stable isotope, pharmacokinetics, pregnancy*

Introduction

Pharmacokinetic studies are conducted in order to determine drug absorption, distribution, metabolism and excretion. This knowledge serves to help determine the appropriate and timely use of medications. This is also an important step in providing individualized therapeutics according to patient characteristics, such as disease state and genotypes of drug metabolizing enzymes. Presented here is an innovative way of conducting pharmacokinetic research in pregnancy, specifically with thyroxine.

Thyroxine (T4) is both a hormone and a therapeutic drug, synthetically produced as levothyroxine (LT4) for the treatment of hypothyroidism – underactivity of the thyroid gland. Thyroxine is essential for normal neural development of the fetus. Clinical evidence indicates that increased doses of T4 are often necessary in early pregnancy, yet it is unclear exactly when to start increasing the dose and how much to supplement. To study the drug's pharmacokinetics, conventional methods of analysis cannot differentiate between the administered drug and endogenous thyroxine. Furthermore, all pharmacokinetic studies are more complex and difficult to conduct during pregnancy, when the use of radiolabeled isotopes or ingesting high doses of the hormone are unethical.

Carbon-13 (^{13}C) is a stable isotope of carbon. It is non-radioactive (thus "stable") with a half-

life, if any, of greater than 0.5 billion years. It possesses no harmful or radiation-related effects. Tests have shown stable isotopes to be safe in newborn infants, and they are also safe in pregnant women. Stable isotopes have been used for over 30 years in studies with infants, children, and adults. The $^{13}C_6$-LT4 derivative of LT4 is highly stable and is not converted to the $^{12}C_6$-LT4 analog, i.e., the prescribed levothyroxine.

MATERIALS AND METHODS

The research protocol was approved by the Georgetown University institutional review board and written informed consents were obtained from all study participants. All women were recruited from Georgetown University Medical Center, where the studies were also conducted.

Hypothyroid women, rendered euthyroid by LT4 replacement, were recruited during pregnancy to participate in the study. Subjects were included in the study if they met the following criteria: women 18 years of age or older at time of consent, able to give written informed consent, euthyroid (LT4-treated hypothyroid) with no other serious illness, prescribed LT4 by their physician for therapeutic reasons during a pregnancy, and anticipating continuing LT4 medication post partum as prescribed by their physician. Subjects were excluded from the study for any of the following reasons: baseline hematocrit lower than 28.0%, TSH higher than 4.5 mIU/L, kidney dysfunction, or ingestion of any other drugs that affect the thyroidal axis, such as those that can alter TSH and thyroid hormone secretion, transport or metabolism.

Subjects were admitted into the General Clinical Research Center on the first day of each study period ("study day"). For each subject there were two study periods—one pre-delivery, then one within three to twelve months post partum, when maternal metabolism returns to normal. Only the LT4 dose on each study day was replaced by ^{13}C-LT4. The women remained on site for about 12 hours. On each of the following days of the pharmacokinetic study period (120 hours), the subjects continued taking the daily dose of their own LT4 (not the study $^{13}C_6$-LT4 preparation).

Participants were required to fast (other than water) for at least five hours prior to, and two hours following, the ingestion of the morning $^{13}C_6$-LT4 dose. They did not take any other medication within two hours of ingesting the study drug, to prevent any interference with the $^{13}C_6$-LT4 rate of absorption, and ate a simple breakfast based on eggs, toast, fruit, yogurt and tea, provided at the Research Center. The women were requested to discontinue taking any iron-containing multivitamins or pills at least one week before and throughout the study. All women were given their prescribed maintenance dose on the first day of the pharmacokinetic study. The subjects kept a record of the times and doses of their own LT4 taken daily during the study period.

The methods for the measurement of thyroid hormone and $^{13}C_6$-LT4 were developed in the Georgetown Bioanalytical Core Laboratory.[1-4] Blood samples were drawn before dose administration (0 h) and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96, and 120 h post dose. At most times, blood was drawn at the subject's home on Days 2, 3, 4, 5, 6, 7, and 14 by a study nurse.

The $^{13}C_6$-LT4 derivative was synthesized and purchased for this study from IsoSciences, LLC. 70 mcg and 100 mcg capsules of study material were prepared by a specialty compounding pharmacy, and the thyroxine content of the compounded capsules verified by HPLC analysis. The $^{13}C_6$-LT4 capsules were kept at controlled and monitored temperature and humidity until use. Quality control potency and stability testing were conducted biannually by Eagle Analytical Services, a specifically designated and independently licensed quality control laboratory. Any LT4 dose that was not a multiple of 70 or 100 mcg was supplemented by the Georgetown research pharmacists with a complementary dose of non-$^{13}C_6$-LT4 levothyroxine to total the subject's dose, while providing the highest possible portion of the dose as the stable isotope study material. For example, a woman prescribed a daily LT4 dose of 225 mcg received 210 mcg of $^{13}C_6$-LT4 (3 x 70 mcg) and an additional 12.5 mcg $^{12}C_6$-LT4 (half of a 25 mcg tablet), reflected in Table 1 as receiving 210 mcg for this study. Note that the results outlined in Table 2 reflect only the $^{13}C_6$-LT4 doses.

TABLE 1 $^{13}C_6$-LT4 Pharmacokinetic Profile for a Single Pregnant Subject

Sample #	Time (h)	$^{13}C_6$-LT4 (ng/mL)	Total T4 (mcg/dL)	Total T3 (ng/dL)
1	0.0	0.000	8.8	155
2	0.5	0.019	9.9	134
3	1.0	0.044	10.3	192
4	1.5	0.059	10.3	119
5	2.0	0.086	8.5	143
6	2.5	0.125	9.1	119
7	3.0	0.141	9.9	178
8	4.0	0.208	10.4	124
9	6.0	0.296	9.7	135
10	8.0	0.461	8.7	136
11	10	0.374	9.3	120
12	12	0.322	8.8	136
13	24	0.196	9.9	133
14	48	0.090	8.9	125
15	72	0.046	8.9	127
16	96	0.029	8.8	113

Serum concentrations of $^{13}C_6$-LT4 and T4 were measured using a validated, sensitive, and specific isotope dilution tandem mass spectrometry.[4] Pharmacokinetic assessments were conducted by standard two-stage approach, using non-compartmental techniques in WinNonLin (Version 5.1.1, Pharsight Corporation, Mountain View, CA). Calculated pharmacokinetic variables included peak concentration (C_{max}), time to peak concentration (T_{max}), $AUC_{0-\infty}$, clearance rate (defined as the ratio of dose administered and AUC), the apparent plasma terminal rate constant (λ), and the half-life of the terminal disposition phase ($t_{½}$) estimated by $\ln(2)/\lambda$. A combination of linear trapezoidal method during the ascending phase and log linear method during the descending phase was used for estimating AUC. Levothyroxine pharmacokinetic parameters in pregnant vs. non-pregnant women were compared using Wilcoxon signed-rank test using significance level of $\alpha = 0.05$. The challenges were (1) to use a traceable form of LT4, and (2) the measurement of very low concentrations of T4, since this study design followed only a single dose of administered ^{13}C-LT4.

Pharmacokinetic Studies of Levothyroxine

The 9 women, whose data is reported here, were recruited during pregnancy. Their ages were 31 to 42 years, and all were euthyroid following LT4 replacement. The women were in various trimesters and were Caucasian (n=7), with Asian (n=1) and African American (n=1), (Table 1). To date we have recruited 16 subjects and are continuing the study. Target therapeutic TSH levels are maintained at around 1 mIU/L, and are trimester-specific during pregnancy. Of the 9 study subjects, 6 returned to participate in the post partum portion of the study approximately 3 to 12 months following delivery.

RESULTS

We observed a peak $^{13}C_6$-LT4 concentration (T_{max}) approximately 8 hours following $^{13}C_6$-LT4 dosing, much later than the previously reported 3 hours for LT4 T_{max}. Table 1 shows the blood level profile for one pregnant subject (data shown to Day 4), with peak $^{13}C_6$-LT4 level achieved at 10 hours.

Median LT4 pharmacokinetic parameters for our study subjects are summarized in Table 2 and again show that the median time to peak blood level at 8 hours. The maximum concentration is similar between the pregnant and non-pregnant groups, but the AUC and clearance rates are significantly different. The percentage change in $^{13}C_6$-LT4 AUC_{0-72} was compared between pregnant and non-pregnant women. It was found that the AUC was almost 50% higher in the women when they were at the pregnant state, compared to these same women postpartum.

The relationship between gestation week and the ratio of AUC in the pregnant vs. non-pregnant state is shown in Table 3, where an increase in the AUC with progression of pregnancy is demonstrated.

TABLE 2 $^{13}C_6$-LT4 Pharmacokinetic Measures Following Oral Administration

		T_{max} (h)	**C_{max} (ng/mL)**	**AUC_{0-72} (ng h/mL)**	**Clearance (L/h)**
Pregnant (n=9)	median	8.0	0.9	14.8	4.5
Non-pregnant (n=6)	median	8.0	0.7	10.5	7.0
AUC = area under the curve					

TABLE 3 $AUC_{13C\text{-}LT4}$ Relative to Week of Gestation

Week of Gestation	**$AUC_{13C\text{-}LT4}$ pregnant/non-pregnant**
13	1.03
16	1.11
22	1.33
25	1.11
30	2.50
36	4.15

DISCUSSION

We successfully conducted pharmacokinetic studies of a single dose of stable isotope-labelled LT4 ($^{13}C_6$-LT4), at patient-specific doses, in non-pregnant and pregnant women serving as their own controls.

The $AUC_{0-\infty}$ results were significantly higher during pregnancy than in the same woman approximately 6 months post partum. The increase in LT4 AUC during pregnancy could be attributed to a decrease in LT4 clearance.

It should be noted that LT4 is not metabolized by the cytochrome family of enzymes, where the activity of certain isozymes can change over the course of a pregnancy. LT4 is metabolized by deiodinases, the activity of which varies in each tissue. These preliminary results suggest that LT4 pharmacokinetics change significantly with gestational age. Additionally, there is a large variability in the pharmacokinetics of LT4 in pregnant women, and a relatively narrower range of variability in non-pregnant women.

Future Research

In order to solidify these findings, a larger group of patients is required. We are continuing to recruit patients, and are currently at n = 16. The mechanisms responsible for the gestational differences in pharmacokinetics need to be investigated. Whether these differences should necessitate dosing schedule changes during pregnancy should also be investigated further.

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Conflict of Interest

The author declares no conflict of interest. Dr. Offie Soldin reports having served as a consultant for Abbott Laboratories.

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