

Stability of levothyroxine sodium 0.4 µg/mL in 0.9% sodium chloride injection

Context—Intravenous levothyroxine therapy decreases vasopressor requirements and prevents cardiovascular collapse in hemodynamically unstable patients eligible for organ donation. The stability of levothyroxine when used in this manner is unknown.

Objective—To determine the stability of levothyroxine solution for intravenous use at a concentration of 0.4 µg/mL diluted in 0.9% sodium chloride.

Design—Triplicate sample sets were prepared by reconstituting levothyroxine 200 µg for injection with 5 mL of 0.9% sodium chloride with further dilution in 500 mL of 0.9% sodium chloride. One sample set was protected from light and the other was left unprotected. Both sample sets were stored at room temperature, and samples from each were analyzed for initial concentration and 4, 8, 12, and 24 hours later.

Conclusions—Levothyroxine sodium 0.4 µg/mL in 500 mL 0.9% sodium chloride is stable for 24 hours at room temperature when protected from light. (*Progress in Transplantation*. 2009;19:354-357)

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Notice to CE enrollees:

A closed-book, multiple-choice examination after this article tests your ability to accomplish the following objectives:

1. Identify the role of levothyroxine in organ transplantation
2. Explain stability concerns when levothyroxine is used as a continuous infusion
3. Describe how levothyroxine is mixed and delivered to potential organ donors

Organ transplantation is the preferred treatment for many end-stage organ diseases. However, the number of suitable organ donors is insufficient to meet the demands of organ transplantation. According to the United Network for Organ Sharing Web site,¹ as of April 12, 2009, 101 576 organ transplantations were needed in the United States, but only 30 530 organs

were transplanted in 2007.² Nearly 80% of transplanted organs in 2007 came from deceased donors, which underscores the need to maximize the quality and quantity of organs procured from this population.^{2,3} Because of the hemodynamic, endocrine, and metabolic disorders associated with brain death, many potential organs are lost.⁴ In order to counterbalance hemodynamic instability and prevent cardiovascular collapse, vasopressors are used, often in dosages high enough to render organs unsuitable for transplantation.⁴

Low levels of circulating thyroxine (T₄) are a possible cause of hemodynamic instability in potential organ donors. The proposed mechanism is that subphysiological concentrations of thyroxine lead to a reduction in myocardial energy stores, thus shifting aerobic metabolism to anaerobic metabolism, leading to hemodynamic instability and a need for inotropic support.⁴ In a recent study⁴ on the effects of administration of levothyroxine sodium in brain-dead potential organ donors, researchers reported a statistically significant decrease in vasopressor requirements after patients

received a 20 µg intravenous bolus of levothyroxine sodium, followed by a continuous intravenous infusion of 10 µg/h, continued until the time of organ donation, discontinuation of life support, or severe hypertension. In another study,³ researchers reported a statistically significant increase in the number of organs donated from patients who received levothyroxine. Currently, all 58 organ procurement organizations in the United States use levothyroxine to various degrees.

However, researchers have not examined the stability of levothyroxine sodium as it is used in this setting. Labeling approved by the Food and Drug Administration states that lyophilized levothyroxine sodium must be used immediately after reconstitution with 5 mL of 0.9% sodium chloride and is not to be added to other intravenous fluids.⁵ It also states that the dry product should be protected from light.⁵ A study examining the stability of levothyroxine sodium at a concentration of 100 µg/mL and stored in polypropylene syringes at a temperature of 5°C for 7 days did not demonstrate a loss of potency, suggesting that the package insert's recommendation to use immediately is too restrictive.⁶ The purpose of this study was to examine the stability of intravenous levothyroxine sodium solution at a concentration of 0.4 µg/mL diluted in 500 mL of 0.9% sodium chloride.

Materials and Methods

Preparation of Samples

Two sets of samples were prepared in triplicate for each analysis. Aseptic technique was used to reconstitute each of 2 vials containing 200 µg of levothyroxine sodium (levothyroxine sodium injection, USP, 200 µg/mL; Bedford Laboratories, Bedford, Ohio, lot 1283958) with 5 mL of 0.9% sodium chloride (final concentration, 40 µg/mL). Drug and diluent were then transferred to a bag of intravenous solution containing 500 mL of 0.9% sodium chloride (sodium chloride 0.9% injection, USP, 500 mL; Baxter Healthcare Corporation, Deerfield, Illinois, lot C736041; final concentration 0.4 µg/mL). One bag was wrapped in aluminum foil and placed in a light-protective bag; the other was left unprotected and stored in a room with fluorescent lighting. The solutions were visually inspected for particulate matter and stored at room temperature. Visual inspections and stability assessments were performed immediately after preparation and at 4, 8, 12, and 24 hours. At each time point, 1 mL of solution was withdrawn from the bag and placed in a test tube for analysis.

Competitive Immunoassay

The ADVIA Centaur T4 assay (Siemens Healthcare Diagnostics Inc, Deerfield, Illinois) is a competitive immunoassay that uses direct chemiluminescent technology. Thyroxine in the sample competes with

thyroxine covalently coupled to paramagnetic particles in the solid phase, for acridinium ester-labeled monoclonal mouse antithyroxine antibody in the light reagent. This assay is used to quantify the amount of thyroxine in serum. The system automatically dispenses 25 µL of sample and 50 µL of T3/T4/VB12 ancillary reagent into a cuvette, then dispenses 250 µL of solid phase and 100 µL of lite reagent and incubates the sample for 7.5 minutes at 37°C. The cuvettes are then separated, aspirated, and washed. Next, 300 µL each of acid and base reagent initiate the chemiluminescent reaction. The amount of thyroxine present in the sample is inversely related to the amount of reactive light units detected. Results are reported in micrograms per deciliter. The reportable range of the ADVIA Centaur T4 assay is 0.003 to 0.3 µg/mL.

Preparation of Standard Solutions and Standard Curve

The assay was washed and a 2-point calibration was done with serial dilutions before analysis. The results indicated that concentrations diluted below 0.1 µg/mL are not obtainable due to differences in the sample matrix. Recovery values greater than 0.1 µg/mL are approximately one-half of the true concentration (Table 1).

Data Analysis

The stability of levothyroxine sodium in each sample solution was determined by calculating the percentage of initial concentration remaining at each time interval. Stability was defined as at least 90% retention of initial levothyroxine sodium. The repeated measures analysis of variance (MIXED PROC on SAS 9.1.3, SAS Institute, Inc, Cary, North Carolina) was used for statistical comparisons. The significance level was set at .05 with Bonferroni adjustment for multiple comparisons.

Results

At least 96% of the initial concentration of levothyroxine remained throughout the 24-hour study period in both diluted preparations (Table 2). Repeated

Table 1 Standard solutions and standard curve for validation of assay for nonserum samples

Diluted concentration sample, µg/mL	Actual test result value, µg/mL
0.012	0.0, 0.0, 0.0, 0.0, 0.0
0.025	0.0, 0.0, 0.0, 0.0, 0.0
0.05	0.0, 0.0, 0.0, 0.0, 0.0
0.1	0.043, 0.043, 0.044, 0.043, 0.042
0.2	0.108, 0.11, 0.109, 0.107, 0.109

Table 2 Stability of levothyroxine sodium solution undiluted 40 µg/mL and 0.4 µg/mL in 0.9% sodium chloride

Sample	Concentration, mean (SD), µg/mL, [% remaining] ^a				
	Initial	At 4 hours	At 8 hours	At 12 hours	At 24 hours
Levothyroxine sodium 40 µg/mL	0.233 (0.0002) [58]				
Levothyroxine sodium 0.4 µg/mL in 0.9% sodium chloride, unprotected from light	0.157 (0.0081) [39]	0.159 (0.0085) [101.3] <i>P</i> > .99	0.177 (0.0051) [112.7] <i>P</i> = .14	0.151 (0.0015) [96.2] <i>P</i> > .99	0.187 (0.0093) [119.1] <i>P</i> = .03
Levothyroxine sodium 0.4 µg/mL in 0.9% sodium chloride, protected from light	0.162 (0.0114) [41]	0.194 (0.0092) [119.8] <i>P</i> = .14	0.164 (0.0105) [101.2] <i>P</i> > .99	0.164 (0.0025) [101.2] <i>P</i> > .99	0.181 (0.0046) [111.7] <i>P</i> = .69

^a Mean (SD) values are from duplicate determinations for 3 samples. *P* values are for differences from time 0.

measures analysis of variance revealed significant increases in concentration between time points 0 and 24 hours in the unprotected specimen (*P* = .03), and between time points 4 and 24 hours (*P* = .048) and 8 and 24 hours (*P* = .02). No significant changes in concentration in the light-protected specimen were observed. Upon visual inspection, no particulate matter and no detectable changes in color were apparent at any time point.

Discussion

A calibration curve and a precision/accuracy validation were performed by using multiple concentrations of levothyroxine in 0.9% sodium chloride to test the feasibility of using a competitive serum immunoassay. The test appeared to be accurate but did have some variability, as the assay was designed and validated for serum samples only. The 0.9% sodium chloride diluent fluid does not contain any enzymes or proteins that may interact with levothyroxine in a serum sample. This matrix effect may account for some variability and overall changes in concentration over time of the stability samples in normal saline. As stated, serial dilutions at concentrations greater than or equal to 0.1 µg/mL recovered approximately one-half of the expected result. This information may account for the variability in levels due to the lower sensitivity of the assay when not used with a serum sample.

Despite these observed limitations in the assay, the coefficient of variation was remarkably low and ranged from 1.05 % (at 0.2 µg/mL) to 1.64 % (at 0.1 µg/mL) over the range of linearity. Therefore, this study does demonstrate that levothyroxine sodium solution is stable for at least 24 hours at a concentration of 0.4 µg/mL when admixed with 500 mL of 0.9% sodium chloride, regardless of light protection.

Conclusion

Levothyroxine sodium 40 µg/mL, when admixed with 500 mL 0.9% sodium chloride to a final concentration of 0.4 µg/mL and protected from light, is stable for at least 24 hours at room temperature. Although the exact mechanism of levothyroxine in the deceased organ donor is not elucidated, it continues to be used. The aim of this study was to attempt to answer the unresolved question of its stability for use as a continuous infusion. This stability information may facilitate increased use of levothyroxine in potential organ donors.

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Financial Disclosures

None reported.

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CE Test Test ID 4000.131: Stability of levothyroxine sodium 0.4 µg/mL in 0.9% sodium chloride injection

Learning objectives: 1. Identify the role of levothyroxine in organ transplantation 2. Explain stability concerns when levothyroxine is used as a continuous infusion 3. Describe how levothyroxine is mixed and delivered to potential organ donors

1. What is the standard loading dose of levothyroxine in a brain-dead potential organ donor?

- a. 20 µg
- b. 100 µg
- c. 300 µg
- d. 500 µg

2. Which of the following is not considered a disorder associated with brain death?

- a. Metabolic disorders
- b. Changes in endocrine function
- c. Increased risk of infectious disease
- d. Hemodynamic instability

3. Low levels of circulating T₄ lead to a reduction in which energy stores?

- a. Cerebral
- b. Muscular
- c. Fatty acid
- d. Myocardia

4. Which of the following is an indication that a levothyroxine continuous infusion should be discontinued?

- a. Organ donation
- b. Severe hypertension
- c. Discontinuation of life support
- d. Any of the above

5. In what volume is levothyroxine diluted when administered as a continuous infusion?

- a. 100 mL
- b. 250 mL
- c. 500 mL
- d. 1000 mL

6. Which of the following 2 factors keep levothyroxine stable for 24 hours?

- a. Premixed with normal saline and maintained at 25°C
- b. Premixed with lactated ringers solution and maintained at 25°C
- c. Protected from light and maintained at room temperature
- d. Premixed with normal saline and refrigerated until use

7. Which of the following diluents does not contain enzymes or proteins that may interact with levothyroxine?

- a. Dextrose 5% and 0.45% saline
- b. Dextrose 5% in water
- c. Lactated Ringer solution
- d. Normal saline

8. Which of the following shifts in metabolism can be caused by subphysiologic concentrations of T₄?

- a. Aerobic to anaerobic
- b. Anaerobic to aerobic
- c. Alkalosis to acidosis
- d. Acidosis to alkalosis

9. Infusing levothyroxine during donor management of deceased donors demonstrated a statistically significant decrease in which of the following requirements?

- a. Antihypertensive
- b. Antidysrhythmic
- c. Vasopressor
- d. Vasodilator

10. During donor management, high doses of which of the following may render some organs unsuitable for transplantation?

- a. Vasopressors
- b. Levothyroxine
- c. Insulin
- d. Cytokines

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