The Effects of Oral Levothyroxine Sodium Application on Serum Copper Concentration in Rabbits

Başaran KARADEMİR *

* Department of Internal Medicine, Faculty of Veterinary Medicine, University of Kafkas, Kars - TURKEY

Summary

This study was carried out to determine the effect of oral Levothyroxine Sodium (T₄) applications with oral CuSO₄ (0.1%) on serum copper (Cu) concentration. Forty male New Zealand rabbits (6 month-old, weighing 2.29±0.06 kg) were allocated to four groups. All groups received 0.1% CuSO₄ (*5H₂O) (Copper (II) sulfate pentahydrate M102787) in drinking water with the beginning of the study. T₄ was applied orally to three experimental groups at the doses of 1.67, 3.33 and 6.67 μg/kg-BW/day except control group. Serum Cu measurements were performed by Atomic Absorption Spectrometer equipped with Flame system. Serum Cu levels were increased on the following days and correlated with applied dose of T₄ (r=0.832, P<0.001). The T₄ affected serum Cu level with minimum r²=56.9% and P<0.001. The effect of time was found significant on serum Cu levels (P<0.001). There was also an interaction between time and groups on serum Cu levels (P<0.001). Consequently, T₄ caused an increase in serum Cu level at second day and this increase continued on the following days.

Keywords: Thyroxine, Serum, Cu, Experimental treatment, Rabbit

INTRODUCTION

Diseases or stressful conditions induce fluctuations on serum Cu level. Territories with low Cu in soil and grass also cause animals to suffer from Cu deficiency. Similarly, different time periods of the year may be effective on serum Cu levels. Cu is involved in Cu/Zn-SOD and ceruloplasmin in human, and Cu deficiency may cause poor activation of these enzymes. And this organism may need oral Cu supplementation.

Serum Cu levels are affected by intestinal absorption, urinary excretion and metabolism rate. It is reported that the intestinal absorption of Cu is limited. Absorption rate of Cu in diet is known as 5-10% for adult animals and 15-30% for young. Therefore, improved Cu absorption gained importance. Active transport mechanism is important for intestinal Cu absorption. This mechanism needs energy and is related to the
thyroidal hormones (TH)\textsuperscript{20,21}.

TH status influences a number of functions in the body including energy metabolism\textsuperscript{21-26}. Thyroxin is a relatively inactive form of thyroidal hormones and used in the clinical practice in patient with hypothyroidism or extirpation of thyroidal glands\textsuperscript{21,25,27}.

Intestinal Cu absorption increases as the Cu requirements also increase\textsuperscript{19}. Increased serum Cu levels in a short time are desired for effective therapy. Copper deficiency usually results from malabsorptive disorders\textsuperscript{28}. Thyroidal hormones can affect absorption, excretion and metabolism of serum Cu. Therefore the aim of this study was to investigate the effect of Levothyroxine sodium with oral CuSO\textsubscript{4} (0.1%).

**MATERIAL and METHODS**

**Animals and Procedures**

The study was approved Ethics Committee of University of Kafkas (Approval No. 2009-21). Clinically healthy 40 male New Zealand Rabbits, aged 6 months and weighing 2.29±0.06 kg were used in this study. The animals were divided into four equal experimental groups. Levotiroksin Sodyum (T\textsubscript{4}) (Levatiron tablet\textsuperscript{®}) orally applied and treatment groups were as follows; Group A was kept as control and received tap water only, Group B received T\textsubscript{4} at 1.67 µg/kg-BW/day, Group C: 3.33 µg/kg-BW/day and Group D: 6.67 µg/kg-BW/day in tap water for four days. T\textsubscript{4} applications were given at ten o’clock daily at a single dose per day. 0.1% Copper (II) sulfate pentahydrate (CuSO\textsubscript{4}·5H\textsubscript{2}O, Merck 102787) in drinking water were given ad libitum to all groups including group A. The applications of T\textsubscript{4} and Cu were given simultaneously at the same time.

The animals were fed with a commercial animal food. The food and water were given ad libitum before and during the experiment. Copper content from food and water was determined by flame system Atomic Absorption Spectrometer (FAAS) (Thermo Elemental S4, Thermo Electron Corporation, Cambridge, UK)\textsuperscript{29,30} and the results were as follows 12.13 mg/kg in dry matter (DM) of food and 0.013 mg/L of tap water.

The commercial food was purchased from Bayramoğlu Yem ve Un San. Tic. A.Ş, ISO 9001:2000, ISO 22000:2005 and the composition is given in Table 1.

**Blood Collections and Laboratory Analyses**

Two milliliters of the blood was collected via cardiac puncture under the ether anesthesia. Serum was separated by centrifugation at 3500 rpm for 15 min and serum Cu contents were also determined by FAAS\textsuperscript{29,30}. Standard solutions for Cu were purchased from Fluka Chemie GmbH, Switzerland (Fluka 61147).

Accuracy Control of FAAS was performed using a previously known standard solution for Cu measurement. This solution was aspirated for 6 times per 10 samples during analyses and the Cu levels were measured. Coefficient of variation (CV) for Cu was calculated from these measured levels. CV for Cu was found to be 3.47%\textsuperscript{29,30}. All lab-ware used were made of PTFE material in the laboratory.

**Statistical Analysis**

The effect of time and interactions between time and T\textsubscript{4} dose on serum Cu level analyzed by repeated measurements ANOVA. One-Way ANOVA were performed for comparisons of days in each T\textsubscript{4} groups (groups A, B, C and D). One-Way ANOVA were also used to compare the serum Cu levels between groups in days (for first, second, third, fourth and last days). Duncan test for all ANOVA analyses was employed for Post Hoc Multiple comparisons between groups\textsuperscript{31}. Pearson correlation test was used to determine the relationship between the dose of T\textsubscript{4} and individual average values of serum Cu levels of days. Correlations between groups receiving four different doses of T\textsubscript{4} (group A, B, C and D) were also analyzed by Pearson correlation test. Linear regression analyses were employed for the effect of T\textsubscript{4} on serum Cu levels. Statistical analyses were performed using SPSS statistical software version 10.0.1\textsuperscript{32}. Data were presented as means ± S.E.M.

<table>
<thead>
<tr>
<th>Table 1. Ingredients of diet given to rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet composition</strong></td>
</tr>
<tr>
<td>Dry matter (%)</td>
</tr>
<tr>
<td>Crude protein (%)</td>
</tr>
<tr>
<td>Crude cellulose (%)</td>
</tr>
<tr>
<td>Crude ash (%)</td>
</tr>
<tr>
<td>Acid insoluble ash (%)</td>
</tr>
<tr>
<td>Calcium (%)</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
</tr>
<tr>
<td>NaCl (%)</td>
</tr>
<tr>
<td>Vitamin A (IU/kg)</td>
</tr>
<tr>
<td>Vitamin D3 (IU/kg)</td>
</tr>
<tr>
<td>Vitamin E (mg/kg)</td>
</tr>
<tr>
<td>Metabolic energy (kcal/kg)</td>
</tr>
</tbody>
</table>

* Raw-materials for this composition: Barly, corn, corn chaff, corn gluten, wheat, rye, craff, cottonseed meal, sunflower meal, dicalcium phosphate, vitamin, mineral |

* P<0.05
RESULTS

The serum Cu levels of groups according to time are illustrated in Fig 1. The Cu levels of all groups increased on second day and maintained their levels on the following days. The increase of serum Cu levels were correlated with the dose of T4 applied (r=0.832, P<0.001) (Fig 2). ANOVA test with multiple comparisons employed for analyses of differences between days (groups for days of first-last) for each T4 groups. Except for first day (P<0.001), there were no significant difference among the days for each T4 group (P>0.05) (Fig 1).

Fig 1. The serum Cu levels of groups according to time

Şekil 1. Grupların zamana göre serum Cu düzeyleri

It was observed that serum Cu levels were significantly affected by T4 treatment depending on the dosage (P<0.001). Results of the regression analyses are given in Table 2.

Interactions between time and the dose of T4 applied on serum Cu levels were analyzed by means of Repeated Measurement ANOVA. The differences among groups were significant in time (P<0.001) and there was an interaction between time and the dose of T4 applied on serum Cu levels (P<0.001). According to the Post Hoc test, statistically significant differences were found among all groups (groups A-D) (P<0.001).

Table 2. Regression analyses results to display effect of T4 amount (µg/kg) on serum Cu level

<table>
<thead>
<tr>
<th>Response</th>
<th>Regression Equations</th>
<th>r² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First day serum Cu level</td>
<td>First day = 0.795 - 0.0006 Applied T4</td>
<td>0.0</td>
</tr>
<tr>
<td>Second day serum Cu level</td>
<td>Second day = 1.09 + 0.137 Applied T4</td>
<td>56.9*</td>
</tr>
<tr>
<td>Third day serum Cu level</td>
<td>Third day = 1.19 + 0.132 Applied T4</td>
<td>70.2*</td>
</tr>
<tr>
<td>Fourth day serum Cu level</td>
<td>Fourth day = 1.21 + 0.122 Applied T4</td>
<td>61.4*</td>
</tr>
<tr>
<td>Last day serum Cu level</td>
<td>Last day = 1.22 + 0.122 Applied T4</td>
<td>66.4*</td>
</tr>
</tbody>
</table>

*: P<0.001, Predictor was amount of T4 (µg/kg BW)
Serum Cu levels among groups (A-D) within particular days (first-last days) were significantly different (P<0.05), except for first day (P>0.05) (Fig 1). There were correlations between T4 groups during experimental days (Table 3).

### Table 3. Correlation test results of T4 groups (r), n=50 (10 animals for each group and for 5 days)

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B</td>
<td>0.387**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td></td>
<td>0.338*</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>0.537**</td>
<td>0.596**</td>
<td>0.767**</td>
</tr>
</tbody>
</table>

*: P<0.05, **: P<0.01

#### DISCUSSION

Thyroxine is an important factor for energy metabolism in the body. Intestinal Cu absorption requires energy for active transport. Levothyroxine sodium (T4) applications can affect the intestinal Cu absorption. The aim of this study was to investigate the effect of orally applied T4 on serum Cu levels.

Several literatures indicated that intestinal Cu absorption could be increased by additives or some manipulations. The Cu level was increased in group A which is control group taking only CuSO4 without T4. In our opinion, these results could indicate that 5-30% of dietary Cu is readily absorbed during oral CuSO4 treatment. However, application of increasing T4 elevates serum Cu levels. Increased serum levels of Cu observed in groups B, C and D could be associated with the effect of T4 on intestinal Cu absorption via improving active transport of Cu.

It was reported that low level thyroidal hormones (TH), induced by thyroparathyroidectomy (TPYT), cause increased serum Cu levels. Contrarily, findings of this study showed that T4 fortifications increased serum Cu level. Leblondel et al. reported that, T4 administration, partially decreased the serum Cu level. The possible cause of this difference between the current and the previous study may be due to purge of all thyroidal and para-thyroidal hormones performed in previous study. Other hormones may also affect the serum Cu levels. In addition extra CuSO4 application in drinking water in our study could induce the serum Cu level elevation.

Liver is a major storage and also a central organ for Cu metabolism. Therefore, mineral contend of liver tissue is a good indicator for the mineral status of the organism such as serum levels. In a similar study, Swick et al. reported that addition of 5% Senecio jacobaea into a diet containing 100 µg/g Cu and 100 µg/g Zn cause an increase in Cu and decrease in Zn levels of liver tissue in the rabbit. Senecio jacobaea is a very common wild flower in the family of Asteraceae that is found throughout Europe. In our study, findings showed a similar course for serum Cu level. Serum Cu level was increased with concomitant supplementation of CuSO4 and T4 in the rabbit, as the results of fortified diet with Cu and Senecio jacobaea.

Interactions between minerals are well known. T4 may be effective on other minerals and the levels of these minerals affect the serum Cu level. In this relation, Domellöf et al. reported that Fe supplements are recommended for therapy or fortification. It was also known that Fe supplementation may have inhibitory effect on Cu absorption. However, Dolmellöf et al. reported that no significant effect of Fe supplementation on intestinal Cu absorption was observed. Similarly, the effect of aluminum chloride on tissue Cu level was studied. The results showed that oral aluminum chloride treatment did not affect tissue Cu levels of stomach, intestinal mucosa, kidney and bone. Our findings apparently showed that CuSO4 supplementation with or without T4 increased the serum Cu level, but interaction between other minerals is possible. Therefore, further investigations are needed to clarify this condition.

The applications of both CuSO4 and T4 were started together on first day in this study. As expected there was no statistically significant difference in Cu levels for the first day. After day one, serum Cu levels were increased in all groups, but these increases were correlated to dose of oral T4 applications. In our opinion, the possible cause of this correlation was due to the effect of T4 on active transport system for intestinal Cu absorption.

Average plane courses for all T4 groups were continued forward until the last day. Excretion and absorption of Cu might be balanced by the dose of T4 during treatment days. Because urinary excretion is also under the effect of thyroidal hormones (TH). The effect of T4 on renal system raises the urinary output by affecting the cardiovascular system.

As the results of this study, oral T4 with CuSO4 in drinking water increased serum Cu level in a dose-
dependent manner. In our opinion, probable consequences of this situation as follows;

- T4 is widely used for the therapy of hipo-thyroidial functional disorders. During this cure, the diet can include high or normal levels of Cu. T4 may elevate the serum Cu level and cause Cu intoxication.

- T4 may be used for the therapy of Cu deficiency in a short time.

- Furthermore, in some cases, Cu deficiencies may be a result of thyroxine hormone insufficiency and the fortification of T4 may help to heal serum Cu disorders. In these situations, thyroxine hormone measurements can also be helpful for the Cu insufficiency, diagnosis and therapy.

Further investigations are needed to clarify these conditions.

REFERENCES


27. Page RB, Monaghan RJ, Walker JA, Voss SR: A model of


