Original Article

Effect of levothyroxine on the fibrinolytic system, lipid profile, and plasma glucose in rats

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Running head: Levothyroxine
Abstract

Thyroid hormones exert many effects on the cardiovascular system. Thyroid abnormality enhances atherosclerosis not only through general risk factors (dyslipidemia) but also via a close relationship with hemodynamic parameters; levothyroxine sodium modulates some elements of the fibrinolytic system, though the relationship is not completely clear. The aim of the current study was to investigate the effects of levothyroxine sodium on fibrinolytic parameters such as plasminogen activator (PA) in rat heart, plasma levels of PA and plasminogen activator inhibitor (PAI), plasma glucose, and the serum lipid profile. Rats were given 50 μg 100 g \(^{-1}\) body weight levothyroxine sodium for one week. Rat heart PA activity was significantly greater in the treated animals than in controls. There were no significant differences between the two groups of rats in PA, PAI or plasma glucose. Total cholesterol and LDL-cholesterol levels were lower in sera from the treated group, resulting in decreased LDL/HDL and total cholesterol/HDL-cholesterol ratios. These results suggest that levothyroxine sodium treatment could have clinical value: it raised PA activity in the heart and reduced the blood cholesterol level. It could therefore confer a beneficial effect on cardiovascular disease risk. A more detailed animal study would be required before clinical trials could be considered.

*Key words:* levothyroxine sodium, thyroid hormone, plasminogen activator, cholesterol
Introduction

Thyroid hormone is secreted by the thyroid gland in an inactive form, thyroxine (T4). Production of T4 is regulated by thyroid-stimulating hormone (TSH) released by the anterior pituitary gland, which in turn is under the influence of thyrotropin-releasing hormone produced in the hypothalamus. Under physiological conditions, thyroid hormone has numerous effects on the cardiovascular system, mostly mediated by an intracellular receptor. Several studies corroborate the strong relationship between low plasma thyroid hormone levels and the progression of cardiovascular disease. Slight changes in the thyroxine level within clinically accepted normal levels could influence the burden of heart failure, as quantified by biological surrogates e.g. natriuretic peptide. The mechanisms by which low levels of thyroid hormones could lead to atherosclerosis and its complications, or alternatively to a bleeding tendency, remain controversial. Indeed, these hormones have multiple effects on the cardiovascular system including alteration of lipoprotein levels, effects on the myocardium, and modification of circulating coagulation parameters and impaired fibrinolysis. The relationship between thyroid disorders and hemostasis seems to be more complex. Fibrinolytic parameters are changed during the pathogenesis of clinically relevant haemostatic abnormalities associated with thyroid dysfunctions.

Blood contains an enzymatic system, the fibrinolytic system, one of the main functions of which is to dissolve and disperse fibrin coagula in the blood vessels. Fibrin can be deposited as a consequence of inefficient fibrinolysis. The fibrinolytic system comprises a proenzyme (zymogen), plasminogen, which can be converted to the active enzyme plasmin by the plasminogen activators (PAs), tPA or uPA. The aim of the present study is to assess the effect of thyroid hormone (levothyroxine sodium) treatment on PAs in heart tissue and plasma and on lipid metabolism in the serum.

Materials and Methods

All experiments were approved by the Institutional Animal Care and Use Committee of Chung-Ang University Medical School. Young male Wistar rats weighing 100-150 g were used. The animals were randomly distributed into two groups. Group 1 (N₁ = 5) served as control. Tablets of synthroxine, generic name levothyroxine sodium (Dalimpharm Ltd., Korea), were ground with a mortar and pestle, and 50 μg/100 g body weight were administered in the drinking water to a second group of rats (group 2, N₂ = 5) for one week. The rats were fasted overnight before being sacrificed by cervical dislocation. Blood and heart tissues were collected from the five rats in each group. All the experiments were performed in triplicate.

From one sample of blood, plasma was prepared using sodium citrate as anticoagulant, and PAs, plasminogen activator inhibitors (PAI) and glucose were measured with replicates. Another part of the blood sample was allowed to clot and the serum was collected for spectrophotometric measurements of total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol, using standard kits. Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) cholesterol were calculated using the formula given by Friedwald et al. (1972) (VLDL = TG/5 and LDL = TC – (VLDL + HDL). Heart tissue was homogenized in 0.25 M sucrose using a Potter-Elvehjem homogenizer. PA activity was determined in heart homogenates and plasma.
euglobin fractions\textsuperscript{12} immediately after sacrifice. All assays for PA activity were performed in duplicate in a 96 microwell plate\textsuperscript{13} in a total volume of 140 μl. The assay mixture contained the plasmin substrate D-Val-Leu-Lys-p-nitroanilide at a final concentration of 37 μg/ml, plasminogen (0.2 IU/ml), heat-inactivated liver microsomes (7 μg protein) and 0.1 M Tricine buffer, pH 8.4. The absorbance was read at 405 nm at 1 h intervals using a Titertek Multiscan plate reader. Activity was expressed as international units of standard human tPA. Protein in the test samples was also determined.\textsuperscript{14} The data were analyzed statistically using Student’s t test; results were expressed as mean ±SD; the significance threshold chosen was P<0.05.

**Results**

The physiological importance of thyroid hormone is well known. PA activity was significantly greater in rat hearts treated for one week with levothyroxine sodium (specific activity 7.98±0.64 IU/mg protein) than in the control group (5.78±0.6 IU/mg protein), as shown in Figure 1.

![Figure 1: Effect of levothyroxine sodium on plasminogen activator (PA) activity in rat heart.](image)

Number of animals in each group was five.

Levothyroxine sodium induced no changes in plasma glucose, PA or PAI in male Wistar rats (Table 1).
Table 1: Comparison of plasma concentrations of plasminogen activators (PA), plasminogen activator inhibitors (PAI), and glucose between control and levothyroxine sodium-treated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated</th>
<th>% Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>61.41±8.46</td>
<td>59.99±6.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA (IU/ml)</td>
<td>1.04±0.80</td>
<td>1.56±0.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI (AU/ml)</td>
<td>12.02±1.40</td>
<td>12.29±4.04</td>
<td></td>
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</tr>
</tbody>
</table>

All the values are mean±SD. Number of animals N₁ (control) = N₂ (treated) = 5.

There were significant differences of 20.6% in the total cholesterol content (P<0.02) and 34.5% in LDL-cholesterol content (P<0.05) between the levothyroxine sodium-treated group and the controls. The triglyceride content was higher in the treated group but the overall atherogenic index (LDL/HDL) was below the control value (Table 2).

Table 2: Effect of levothyroxine sodium on lipid metabolism

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated</th>
<th>% Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>124±9.79</td>
<td>98.4±11.2</td>
<td>−20.6</td>
<td>0.0186 **</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>44.35±4.34</td>
<td>38.81±3.30</td>
<td>−12.5</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>34.47±7.31</td>
<td>43.67±11.30</td>
<td>26.7</td>
<td></td>
</tr>
<tr>
<td>VLDL-Cholesterol</td>
<td>06.89±1.46</td>
<td>08.73±2.66</td>
<td>26.7</td>
<td></td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>73.21±14.88</td>
<td>47.96±0.62</td>
<td>−34.5</td>
<td>0.0475*</td>
</tr>
<tr>
<td>LDL/HDL Ratio</td>
<td>1.65</td>
<td>1.23</td>
<td>−25.5</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol/ HDL Ratio</td>
<td>2.79</td>
<td>2.53</td>
<td>−9.3</td>
<td></td>
</tr>
</tbody>
</table>

*P< 0.05; **P< 0.02.
Figure 2: Differences between control group and treated group in total cholesterol, LDL, and HDL after treatment for one week with levothyroxine sodium.

**Discussion**

This experiment also demonstrated that levothyroxine sodium treatment promotes the release of PA from heart, kidney and liver. The tissues with the highest levels of tPA are brain and heart.\(^{15}\)

The specific stimulus for increased PA activity in the heart following thyroid hormone treatment is not known, but it is possible that the cardiac cells are sensitized to catecholamines.\(^{16}\) However, a short-term elevation of thyroid hormones can result in increased myocardial contractility and cardiac output, whereas long-term elevation can result in heart failure.\(^{3}\)

Many reports on PA and PAI levels in the plasma are conflicting. Burgraaf and colleagues found elevated levels of most endothelium-associated proteins in hyperthyroid patients but no evidence that coagulation and fibrinolysis were activated.\(^{17}\) However, Erem and his colleagues found lower levels of tPA and higher levels of PAI-I, suggesting reduced fibrinolytic capacity.\(^{18}\) Other studies have demonstrated that the balance between tPA and PAI is disturbed in favor of PAI-I in hyperthyroid patients, resulting in impaired fibrinolysis.\(^{19}\) Similarly, some studies of hypothyroidism have revealed low tPA and PAI-I activities\(^{20}\) and increased fibrinolytic activity\(^{21}\), whereas others have obtained contrary results, i.e. plasma PAI activity increased.\(^{22}\)

Untreated hypothyroidism is associated with hyperlipidemia, specifically with higher levels of total and LDL cholesterol, which could have serious cardiovascular consequences.\(^{23}\) The reduction in total cholesterol on levothyroxine sodium treatment could be attributed to decreased levels of HDL and LDL-cholesterol.\(^{24}\) Both the LDL/HDL and total cholesterol/HDL ratios decreased after levothyroxine treatment for one week. Our studies support the hypothesis that cardiovascular abnormalities can be corrected by levothyroxine sodium therapy in cases of thyroid failure, as this treatment had modest effects on PA activity and on the total and LDL cholesterol levels in the serum.

**Conclusion**

Levothyroxine sodium is typically used to treat hypothyroidism. It can also be used to treat goiter because it lowers the levels of thyroid-stimulating hormone (TSH). However, our experiments
demonstrated that levothyroxine sodium significantly decreased the blood levels of total cholesterol, LDL-cholesterol and HDL-cholesterol in rats after one week of treatment. These results suggest that levothyroxine sodium could confer a beneficial effect on cardiovascular disease risk. A more detailed animal study would be required before clinical trials could be considered.
References


