Effect of thyroxine on plasminogen activator and inhibitor activity in rat

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Received 20 August 2008; revised 1 December 2008

Thyroid hormones influence mineral metabolism, distribution of water and electrolytes and are therefore of great importance in the maintenance of homeostasis under normal and diseased conditions such as renal failure. The present study was carried out to determine the effect of thyroxine on fibrinolytic parameters such as plasminogen activators (PA) in rat kidney, levels of PA and plasminogen activator inhibitor (PAI), glucose in plasma and serum lipid profile injected with thyroxine (75 µg eltroxine/100 g body weight, ip for 7 days). Treatment increased PA activity significantly in rat kidneys. No changes were seen in PA, PAI and glucose in plasma of rats. There was significant decrease in total cholesterol and LDL-cholesterol levels in serum of treated group resulting in the decrease of HDL/LDL and total cholesterol/cholesterol ratios. However, triglycerides and VLDL showed significant higher activity in the serum of treated group as compared to controls. The results suggest beneficial effects of thyroxine treatment by increasing PA activity in kidney and reducing the cholesterol content in blood. It may be helpful to prevent hypercoagulable state by maintaining the normal homeostatic balance and restoring renal function.

Keywords: Fibrinolytic Parameters, Homeostatic balance, Renal function, Thyroxine

Endocrine disorders can influence the haemostatic balance. Abnormal coagulation has been observed in patients with abnormal hormone levels. The link between haemostatic system and thyroid disease has been known for long but studies on the relationship between thyroid dysfunction and thrombosis are still lacking. Several mechanisms are believed to be involved at different levels\(^1,2\). Hemodynamic alterations in renal injury relate primarily to endothelial cell injury\(^3\). During renal injury the endothelial cell loses its ability to regulate vascular tone, perfusion, permeability and inflammation/adhesion. This loss of regulatory function has a detrimental impact on renal function. Thyroid hormones also regulate renal cell growth and differentiation\(^4\). Both hypo- and hyper-thyroidism are accompanied by numerous abnormal renal functions. Fast short-term thyroid gland reserve in patients on haemo-dialysis was found to be low in comparison to normal group and hypothyroidism was observed in 25% of the hemodialysis patients\(^5\). The kidney plays an important role in the peripheral metabolism of iodine and thyroid hormones, and thyroid function is altered in certain kidney diseases, particularly chronic renal failure. The pathogenesis of these alterations is currently under active investigation.

Kidney has been recognized a major source of plasminogen activators. Urokinase plasminogen activator (uPA) is synthesized by the epithelial cells lining the straight parts of both proximal and distal tubules whereas tissue plasminogen activator (tPA) found in the medulla and cortex of human glomeruli is localized in the endothelial cell lining of glomerular floculus\(^6\). Maximum tPA mRNA abundance is observed in the epithelial cells lining the distal parts of the collecting ducts. PA production and secretion provide an efficient mechanism to prevent tubular obstruction. In absence of a potent proteolytic system in thyroid dysfunction and hypercoagulable state which could lead to renal failure, efforts are underway to improve hemo-dynamics to prevent kidney damage. The aim of the present study is to assess the effect of thyroxine treatment on plasminogen activators in kidney and plasma as well as lipid metabolism in serum.

Bovine plasminogen, standard human tissue plasminogen activator was from American Diagnostica Inc. Greenwich, USA, Synthetic substrate S-2251 was from chromogenex, Sweden. Glucose kit was from J. Mitra and Co. Ltd., New Delhi, India. Triglyceride and HDL-cholesterol kits were from Ranbaxy Laboratories Ltd., New Delhi. Eltroxin was purchased from Glaxo India Ltd., Bombay, India. All other reagents used were of analytical grade and purchased locally

Male Wistar rats (100-150 g) obtained from Central Animal House, Panjab University,
Chandigarh were housed in plastic cages with free access to pellet diet and water. All experiments involving animals were done following guidelines laid down by Animal Ethics Committee Rules and Regulations of the Institute. The rats were divided into two groups of 4 animals each. Group 1 served as control. Rats in group 2 were given daily ip injections of eltroxine (75 µg/100 g body weight) for 7 days as per Dong et al\textsuperscript{7}. Overnight fasted rats were sacrificed by cervical dislocation. Blood and kidney tissues were collected.

Plasma was prepared using sodium citrate as anti-coagulant and used to assay PA, PAI\textsuperscript{8} and glucose\textsuperscript{9}. Total cholesterol\textsuperscript{10}, triglycerides\textsuperscript{11}, HDL\textsuperscript{12}, LDL and VLDL cholesterol\textsuperscript{13} were determined in the serum. Total cholesterol, triglycerides, HDL, LDL and VLDL cholesterol were determined in the serum. Kidney tissue was homogenized in 0.25 M sucrose using a Potter-Elvejem homogenizer. PA activity was determined in kidney homogenates and plasma euglobin fractions\textsuperscript{14}.

**Assay for plasminogen activator (PA) activity:** Assays were carried out in a total volume of 140 µl in 96 microwell plates\textsuperscript{15}. The reaction mixture contained the following: plasmin substrate (S-2251) D-Val-Leu-Lys-p-nitoanilide (37 µg/ml), plasminogen (0.2 IU/ml), heat inactivated liver microsomes as activator (7 µg protein), 0.1 M Tricine, pH 8.4 and test samples (20 µl). Blank incubations contained buffer in place of enzyme. Absorbance at 405 nm was read at 1 hr intervals using a Titertek Multiscan plate reader. Activity was expressed as international units of standard human tPA. Protein estimation in the test samples was also carried out\textsuperscript{16}.

Statistical analysis of data was done using Student’s t test. Results were expressed as mean ± SD, significance was chosen at \( P < 0.05 \).

Thyroid dysfunction is associated with changes in hemo-dymanics and delay in renal cell growth and differentiation. It also influences specific actions on defined tubular segments and permeabilities of membranes\textsuperscript{4}. There are several reports on the hemodymanic alterations primarily due to endothelial cell injury and impaired fibrinolytic activity\textsuperscript{1,17}. Fibrinolytic activity has been predominantly ascribed to plasminogen activators (PA) and proximal tubule of the kidney is a major site of uPA synthesis. The PA activity increased significantly (\( P < 0.001 \)) in rat kidney on thyroxine treatment (15.52±1.04 IU/mg protein, specific activity) for one week as compared to control group (10.46±1 IU/mg protein). Adamson and Ingbar\textsuperscript{18} also suggested that short term treatment of cells or animals with low doses of tri-iodothyronine (T3) can lead to modifications of membrane transport processes, accompanied by an increase in intracellular cAMP. In rat hepatoma cells, cAMP induced stimulation of tPA synthesis has been demonstrated\textsuperscript{19}. Increase in PA activity in kidneys may encourage the removal of intraluminal fibrin or extracellular matrix components obstructing urinary flow.

There was no change in PA, PAI and glucose levels in rat plasma after one week treatment of thyroxine (Table 1). These findings are consistent with the previous reports where fluctuations and variations in the tPA levels has been reported\textsuperscript{20,21}. Some studies of hypothyroidism found low levels of tPA and PAI-1 activities and increased fibrinolytic activity\textsuperscript{22}, whereas others found the opposite results, i.e. increased plasma PAI-1 activity\textsuperscript{23}. Non significant changes in plasma glucose levels were also observed between treated and normal controls. Muller et al.\textsuperscript{24}, have reported that thyroxine treatment increased basal expenditure (+ 8%), glucose disposal (+ 31%) and oxidation (+ 87%) but decreased nonoxidative glucose metabolism (- 30%).

### Table 1—Plasma PA, PAI, glucose levels and serum lipid profile parameters in control and thyroxine treated rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated</th>
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<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>61.41 ± 8.46</td>
<td>67.82 ± 7.06</td>
</tr>
<tr>
<td>PA (IU/ml)</td>
<td>1.04 ± 0.80</td>
<td>1.21 ± 0.71</td>
</tr>
<tr>
<td>PAI (AU/ml)</td>
<td>12.02 ± 1.40</td>
<td>15.40 ± 4.50</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>34.47 ± 7.31</td>
<td>56.89 ± 11.30**</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>124.00 ± 9.79</td>
<td>98.40 ± 11.2**</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>44.35 ± 4.34</td>
<td>38.52 ± 4.59</td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dl)</td>
<td>6.89 ± 1.46</td>
<td>11.37 ± 2.26**</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>73.21 ± 14.68</td>
<td>49.95 ± 8.96*</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>1.65</td>
<td>1.29</td>
</tr>
<tr>
<td>Total cholesterol /HDL ratio</td>
<td>2.79</td>
<td>2.55</td>
</tr>
</tbody>
</table>

\( P \) Values: * \( < 0.05 \); ** \( < 0.02 \).
Thyroid hormones regulate lipid synthesis, their mobilization and catabolism. A significant decrease was seen in the total cholesterol content and LDL-cholesterol content of thyroxine treated group as compared to control group (Table 1). Reduction in total cholesterol due to thyroxine treatment was due to decrease in HDL and LDL-cholesterol. Similar findings have also been reported before. However, triglyceride content increased significantly in thyroxine treated group as compared to control group. In hyperthyroidism, removals of triglycerides are accelerated but simultaneously their production rate is also increased. Since VLDL is the main carrier of triglycerides, the concentration of plasma triglycerides is an evidence of slightly higher VLDL concentration. Although the decrease in HDL-cholesterol was not significant, considerable interest has been focused on the concentrations and proportions of the HDL subclasses. Miller et al., reported that HDL2 subfraction of HDL which bears a negative risk for coronary heart disease (CHD) is increased in hypothyroid patients. The enzyme hepatic lipase (HL) has been known to facilitate cholesterol elimination through its action as a phospholipase acting on HDL2 particle during its passage through the liver. These changes seem to be beneficial as both LDL/HDL ratio and total cholesterol/HDL ratio decreased on thyroxine treatment. In summary, the results of present study suggest that thyroxine treatment improves the haemostatic balance as it increases PA activity in kidney and lowers total and LDL-cholesterol levels and overall atherogenic index ratios.

Thanks are due to Dr. Aditya Shastri, Banasthali University, Rajasthan for facilities.

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