Irbesartan prevents myocardial remodeling in experimental thyrotoxic cardiomyopathy

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Abstract. This study evaluated the effects of irbesartan and propranolol on thyroid hormone (TH)-induced cardiac functional and structural remodeling. A rat model of thyrotoxicosis was established by daily intraperitoneal injections of L-thyroxine (T4, 100 µg/kg) for 4 weeks. Forty Sprague-Dawley rats were randomly divided into four groups (n = 10 each): control group, T4 group (T4 alone), T4 plus irbesartan group (T4-Irb, 30 mg/kg), and T4 plus propranolol group (T4-Pro, 0.5mg/mL of drinking water). Cardiac chamber size and functional parameters were measured by echocardiography and cardiomyocyte diameter. Heart rate (HR) and cardiac fibrosis were determined. T4 alone showed significantly increased HR and cardiomyocyte width (25.0 ± 1.77 μm vs 18.8 ± 0.84 μm, P < 0.001) with fibrosis, reduced left ventricle (LV) longitudinal strain (Slong; -16.0 ± 6.27 vs. -22.7 ± 5.19 %, P < 0.001) compared with control. When compared with T4 alone, T4-Irb showed significantly improved LV Slong (-21.4 ± 1.84 vs. -16.0 ± 6.27 %, P = 0.017) and reduced cardiomyocyte width (21.0 ± 1.0 μm vs. 25.0 ± 1.77 μm, P = 0.002) with comparable HR (458.4 ± 24.3 vs. 486.6 ± 30.1 bpm, P = 0.086). However, T4-Pro showed significantly reduced HR with improved LV Slong without alteration of cardiomyocyte width and fibrosis compared with T4 alone. In conclusion, renin-angiotensin system (RAS) blocking by irbesartan could significantly attenuate TH-induced cardiac structural and functional remodeling. However, HR reduction by propranolol could not alter structural remodeling, which may implicate the RAS as having an important role in thyrotoxic cardiomyopathy beyond tachycardia.

Key words: Cardiomyopathy, Heart failure, Thyrotoxicosis, Renin-angiotensin system, Echocardiography

HYPERTHYROIDISM is a common endocrine disorder that has important cardiovascular manifestations associated with remarkable changes in cardiac structure and function [1]. Clinically, the effects of excess thyroid hormone (TH) on the cardiovascular system translate into a wide variety of signs and symptoms, ranging from sinus tachycardia to severe left ventricular (LV) dysfunction and heart failure [2, 3]. The effects of TH on the heart are exerted both directly at the cellular level and by indirect interactions with the renin-angiotensin system (RAS), sympathetic nervous system (SNS) [2] and vascular smooth muscle [4, 5], which lead to increased plasma volume, cardiac preload and, finally, cardiac output [1]. Although enhanced cardiac function is the most recognized outcome of hyperthyroidism, heart failure can occur even in young patients with hyperthyroidism in the absence of known heart disease and atrial fibrillation (AF) [6-8]. A recent study indicated decline cardiac function that begins after 4 weeks of TH treatment [9]. Interestingly, restoring normal levels of TH often reverses the cardiac dilatation and dysfunction, making hyperthyroid cardiomyopathy...
a potentially curable form of heart failure [8]. However, whether cardiomyopathy in hyperthyroidism is due to direct toxic effects of the TH or due to tachycardia-induced is still the subject of ongoing debate [10].

Based on the clinical findings of hyperthyroidism such as increased heart rate, cardiac output, and enhanced myocardial contractility, hyperthyroidism is a hyperadrenergic state that may cause myocardial hypertrophy [11]. However, there are also conflicting reports regarding the preventative nature of sympathetic inhibition in TH-induced cardiac hypertrophy [12, 13]. TH directly stimulates renin mRNA in vivo [13, 14] and in vitro [15], and increases both the synthesis and secretion of angiotensinogen and angiotensin (ANG) II receptors [16]. Also, TH activates circulating and tissue RAS without involving the SNS, and this may account for the cardiac hypertrophy observed in hyperthyroidism [14, 17]. These findings suggest that TH-induced cardiac hypertrophy could be involved in changes in circulating RAS or SNS activity, and these remodeling would be the cause of heart failure. However, it has yet to be established whether the impact of high output state with augmented HR or activated RAS contributes to TH-induced heart failure. This study investigated the effects of irbesartan, a RAS blocking agent, and propranolol, which blocks β-adrenoceptors, on TH-induced cardiac functional and structural remodeling by strain echocardiography and pathological analysis.

**Materials and Methods**

**Animal models**

Animal procedures were performed according to National Institutes of Health guidelines. The study protocol was approved by the Ethical Committee of Pusan National University Hospital. Healthy male Sprague-Dawley rats weighing 180-200 grams were obtained from the Japan SLC (Hamamatsu, Japan). Animals were housed in plastic cages (five animals each) with ad libitum access to water and standard rat chow. They were maintained under standard laboratory conditions (controlled temperature of 21-24°C, controlled light cycle consisting of alternating 12 hour periods of light and darkness) during all experiments. Levo-thyroxine (T4) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Blood pressure was assessed by a tail-cuff in conscious rats before each experiment.

**Experimental design**

A rat model of thyrotoxicosis was induced by daily intraperitoneal injections of T4 (100 µg/kg/day) for 28 days as previously described [14]. Forty Sprague-Dawley rats were randomly divided into four groups (n=10 each): control group, T4 group (T4 alone), T4 plus irbesartan group (30 mg/kg/day irbesartan, T4-Irb) and T4 plus propranolol group (0.5mg/mL of drinking water, T4-Pro). Control rats received daily intraperitoneal administration of normal saline. Irbesartan was administered in drinking water once a day during the 28 days of treatment. At the end of the treatments, serum T3, free T4 and thyroid stimulating hormone (TSH) concentrations were measured using a competitive inhibition enzyme immunoassay kit (CUSABIO Biotech, Hubei, China). All rats underwent strain echocardiography and cardiac chamber size measurements, and hearts were removed. In the five rats in each group, hearts were sectioned transversely and slices were snap-frozen or immersion-fixed in 10% formalin for pathological analysis. Isolated cell aliquots were fixed in glutaraldehyde (myocyte length measurements, 40 cells/heart), then cardiomyocyte diameter and cardiac fibrosis were measured.

**Hemodynamic parameters**

In all rats, body weight (BW), systolic blood pressure (SBP) and heart rate (HR) were measured weekly at approximately the same time of day by tail-cuff plethysmograph (Kent Scientific, Orlando, FL, USA). Rats were familiarized with the apparatus for a total of 7 days before measurements were taken.

**Echocardiography**

Echocardiography was performed in animals just before they were sacrificed. Animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The chest was shaved, and the animals were placed on a warming pad to maintain normal body temperature. Echocardiographic gel was applied to the left hemithorax and examination was performed using a 15 MHz transducer on a Vivid 7 Dimension ultrasound apparatus (General Electric, Horten, Norway). The transducer was lightly placed on the left hemithorax and two-dimensionally targeted M-mode echocardiograms were obtained from short-axis views of the left ventricle (LV) at the level of the papillary muscle tips and apical 4-chamber view with a frame rate of 250-300/s. LV end-diastolic septal and posterior wall thickness (IVSd and PWTd, respectively), LV end diastolic dimension (LVEDD) and
LV end systolic dimension (LVESD) were measured. The LV fractional shortening (FS) was calculated according to the following formula: FS (%) = (LVEDD - LVESD) / LVEDD. The mitral inflow velocity was measured by pulsed wave Doppler with a sample volume 2 mm from the apical 4-chamber at a sweep speed of 100 mm/s. The following measurements of LV diastolic function were determined: peak early (E) and late (A) diastolic mitral flow velocity and deceleration time of the E wave (DT), peak early (Ea) diastolic mitral annular velocity.

Strain echocardiography

Three heart beats were collected from each view and LV images sampled at appropriate frame rate of 80-150/s were post processed using the speckle tracking echocardiography software (Echopac 6.0.0; General Electric). Briefly, the displacement of speckles of LV myocardium (the acoustic markers) in each spot was tracked from frame-to-frame in each of the apical views. The software package then automatically tracked the motion through the rest of the cardiac cycle, providing the LV strain (%) profile by default. Peak LV longitudinal strain ($S_{long}$) was collected from the apical 4-chamber view (Fig. 1A). The peak systolic LA strain was obtained from the basal segment of the inter-atrial septum in the apical 4-chamber view by the tissue Doppler strain (TDI) (Fig. 1B). For the longitudinal measurements, a computation area of 1 x 0.5 mm with an elliptical shape was chosen.

Heart preparation

After echocardiography, the thorax of each animal was opened. Cardiac arrest was induced by injection of 14 mM potassium chloride. The heart was then quickly removed, blotted and weighed. The ventricles were carefully dissected, weighed, fixed in 10% neutral formalin and prepared for microscopic analysis.

Microscopic studies

The left ventricle was transversely sectioned at the midportion between the apex and base. A cross-sectional slice was removed and fixed in 10% neutral formalin at room temperature for 48-72 h. The slice was dehydrated in an increasing alcohol series, immersed in benzene for 20 min, and embedded in paraffin. All the microscopic fields were examined, and width of cardiomyocytes was measured using ocular micrometer. Five hundred cardiomyocytes were examined in five different fields. Apoptosis and necrosis, presence of fat droplet and inflammation of cardiomyocytes were evaluated on hematoxylin and eosin stain findings. To identify possible interstitial fibrosis, Masson-Trichrome staining was carried out in a NexES Special Stainer (Ventana Medical Systems, Tucson, AR, USA). Each 4 µm-thick section from five rats in each group was put in the stainer and Trichrome Blue kit was applied (Ventana Medical Systems).

Statistical analyses

The statistical analyses were performed using the SPSS version 15.0 package program (SPSS, Chicago, IL, USA).
Numeric data are expressed as the mean ± SD. Chi-square test for testing differences of categorical variables among four groups was performed. Statistical analysis of four continuous variables among four groups was performed using ANOVA, and \( P \) values < 0.05 were accepted as statistically significant. Turkey’s post hoc test was used to make individual comparisons between groups when a significant change was observed with ANOVA.

### Results

**Induction of thyrotoxicosis**

Administration of T4 for 4 weeks significantly increased serum free T4 and T3, HR and the ratio of heart weight (HW) to BW. The levels of T3 and free T4 were significantly increased with T4 administration (T4 alone; 25.3 ± 1.96 ng/mL and 5.62 ± 0.09 ng/dL, T4-Irb; 24.2 ± 0.72 ng/mL and 5.55 ± 0.10 ng/dL, T4-Pro; 24.9 ± 0.77 ng/mL and 5.48 ± 0.08 ng/dL vs. control; 5.5 ± 0.83 ng/mL and 2.78 ± 0.06 ng/dL, all \( P < 0.05 \)). Also, the levels of TSH were significantly decreased by T4 administration (T4 alone; 0.72 ± 0.16 μU/mL, T4-Irb; 0.77 ± 0.14 μU/mL, T4-Pro 0.75 ± 0.12 μU/mL vs. control; 3.34 ± 1.26 μU/mL, \( P < 0.05 \)). Although TSH was not sufficiently suppressed, T4 administration for 4 weeks was effective at producing thyrotoxicosis in this model as previously described [14].

**Hemodynamic parameters**

BW, HR and SBP at the beginning were the same in all groups of rats (Table 1). After 4 weeks, T4 alone showed significantly increased HR, HW and the HW/BW ratio and decreased BW compared with control (all \( P < 0.05 \)) (Table 1). When compared with T4 alone, T4-Irb showed significantly reduced HW/BW (2.39 ± 0.36 vs. 2.87 ± 0.68, \( P = 0.032 \)) and SBP (130.5 ± 20.2 vs. 143.9 ± 14.3 mmHg, \( P = 0.043 \)) in spite of similar HR (458.4 ± 24.3 vs. 486.6 ± 30.1 bpm, \( P = 0.086 \)). However, T4-Pro showed no significant differences in HW and HW/BW with significant reduction of HR compared with T4 alone (385.2 ± 36.7 vs. 486.6 ± 30.1 bpm, \( P < 0.001 \)).

Echocardiography

**Ventricular geometric patterns**

The results of 2-dimensional echocardiographic examination are shown in Table 2. T4 alone showed significantly increased LVESD, IVSd and LA diameter (Fig. 2) without change of LVEDD, PWTd and relative wall thickness (RWT) compared with control. Interestingly, there were two rats in the T4 alone that displayed significantly increased LVEDD and LVESD (7.4 mm and 7.27 mm in LVEDD; 5.45 mm and 5.34 mm in LVESD). Although T4-Irb showed significant decrease of LVESD and LA diameter compared with T4 alone group, there was no significant difference in LV geometrical parameters between T4-Pro and T4 alone (Table 2).

**Ventricular functional changes: Doppler strain echocardiographic findings**

T4 alone showed significantly lower ejection fraction (EF) and FS compared with control, but within normal range (Table 2). Also, peak systolic mitral annulus velocity (Sm) and LV S_long was significantly impaired in T4 alone compared with control (Table 3). The two rats with significantly increased LV internal dimension showed markedly reduced EF and FS (57%, 58 % in EF and 26%, 28% in FS, respectively). Other eight T4 rats showed more deteriorated S_long (-16.32 ± 4.13%) despite comparable systolic function measured by EF (74.32 ± 3.67%) and FS (37.54 ± 2.21%) with control. T4-Irb showed significantly improved LV S_long compared with

### Table 1 Changes in heart rate, heart weight and body weight

<table>
<thead>
<tr>
<th></th>
<th>Control (n=10)</th>
<th>T4 alone (n=10)</th>
<th>T4-Irb (n=10)</th>
<th>T4-Pro (n=10)</th>
<th>( P ) (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline BW (gram)</td>
<td>207.3 ± 4.74</td>
<td>208.00 ± 3.12</td>
<td>207.3 ± 2.11</td>
<td>207.8 ± 2.87</td>
<td>0.910</td>
</tr>
<tr>
<td>4 weeks BW (gram)</td>
<td>405.1 ± 29.0</td>
<td>342.0 ± 38.6*</td>
<td>369.8 ± 18.6*</td>
<td>378.0 ± 18.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4 weeks HW (mg)</td>
<td>910.5 ± 32.4</td>
<td>892.3 ± 56.8*</td>
<td>883.4 ± 51.3</td>
<td>982.3 ± 59.3*</td>
<td>0.004</td>
</tr>
<tr>
<td>HW/BW (mg/g)</td>
<td>2.25 ± 0.87</td>
<td>2.87 ± 0.68*</td>
<td>2.39 ± 0.36*</td>
<td>2.60 ± 0.30*</td>
<td>0.003</td>
</tr>
<tr>
<td>Baseline HR (bpm)</td>
<td>398.7 ± 20.5</td>
<td>401.3 ± 29.2</td>
<td>405.8 ± 25.7</td>
<td>397.5 ± 32.6</td>
<td>0.789</td>
</tr>
<tr>
<td>4 weeks HR (bpm)</td>
<td>408.3 ± 33.3</td>
<td>486.6 ± 30.1*</td>
<td>458.4 ± 23.4*</td>
<td>385.2 ± 36.7*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline SBP (mmHg)</td>
<td>138.8 ± 6.9</td>
<td>139.8 ± 7.6</td>
<td>138.2 ± 7.9</td>
<td>139.2 ± 10.8</td>
<td>0.687</td>
</tr>
<tr>
<td>4 weeks SBP (mmHg)</td>
<td>142.3 ± 7.1</td>
<td>143.9 ± 14.3</td>
<td>130.5 ± 20.2*</td>
<td>138.4 ± 22.4</td>
<td>0.045</td>
</tr>
</tbody>
</table>

All values are presented as the mean ± SD; n, number of rats; T4, L-thyroxine; Irb, irbesartan; Pro, propranolol; BW, body weight; HW, heat weight; HR, heart rate; SBP, systolic blood pressure; *\( P < 0.05 \) vs. control; †\( P < 0.05 \) vs. T4 alone; ‡\( P < 0.05 \) vs. T4 Pro
T4 alone and there were no significant differences in Sm and LV S_long between control and T4-Irb. T4-Pro showed no differences in Sm with significant improvement of LV S_long compared with T4 alone (Table 3).

In terms of diastolic functions, although there was no significant difference in peak E velocity, peak A velocity was significantly increased in T4 alone, T4-Irb and T4-Pro compared with control (Table 3, Fig. 3). Prolongation of DT (95.6 ± 3.06 vs. 82.0 ± 5.39 ms, P = 0.026), reduction of Ea velocity (4.34 ± 1.36 vs. 6.06 ± 1.74 cm/s, P < 0.001) and increase of E/Ea (21.5 ± 6.37 vs. 15.0 ± 6.87 cm/s, P = 0.003) were significantly prominent in T4 alone compared with control (Fig. 3C). T4 alone showed significantly enlarged LA diameter (3.83 ± 0.11 vs. 3.45 ± 0.23 mm, P = 0.019) and markedly reduced mean peak systolic LA strain (13.9 ± 2.38 vs. 21.5 ± 2.63 %, P < 0.001) compared with control. T4-Irb and T4-Pro showed reduced LA diameter and E/Ea with improved mean peak systolic LA strain compared with T4 alone, but showed still more reduced LA strain values compared with controls (Tables 2 and 3).

**Morphological Data**

**Cardiomyocyte analysis**

Measurements of transverse fiber diameter (myocyte width) ranged from 18 μm (Control) to 27.5 μm (T4 alone) (Table 4). T4 alone promoted a significant increase in myocyte width compared with control (25.0 ± 1.77 vs. 18.8 ± 0.84 μm, P < 0.001). However, T4-Irb showed a significant decrease in myocyte width (21.0 ± 797.6x797.6
Table 3 Parameters of doppler echocardiography and strain echocardiography

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10)</th>
<th>T4 (n=10)</th>
<th>T4-Irb (n=10)</th>
<th>T4-Pro (n=10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E, cm/s</td>
<td>78.4 ± 11.3</td>
<td>75.1 ± 27.1</td>
<td>73.0 ± 18.6</td>
<td>74.9 ± 13.5</td>
<td>0.061</td>
</tr>
<tr>
<td>A, cm/s</td>
<td>40.4 ± 12.5</td>
<td>81.5 ± 14.8*</td>
<td>67.6 ± 19.5*</td>
<td>70.63 ± 13.5*</td>
<td>0.021</td>
</tr>
<tr>
<td>DT, ms</td>
<td>82.0 ± 5.39</td>
<td>95.6 ± 3.06*</td>
<td>83.1 ± 7.21*</td>
<td>88.4 ± 4.47*</td>
<td>0.034</td>
</tr>
<tr>
<td>Ea, cm/s</td>
<td>6.06 ± 1.74</td>
<td>4.34 ± 1.36*</td>
<td>5.48 ± 0.80</td>
<td>5.24 ± 0.84</td>
<td>0.033</td>
</tr>
<tr>
<td>E/Ea</td>
<td>15.0 ± 6.87</td>
<td>21.5 ± 6.37*</td>
<td>17.8 ± 6.08*</td>
<td>18.8 ± 6.46*</td>
<td>0.002</td>
</tr>
<tr>
<td>Sm, cm/s</td>
<td>4.74 ± 1.72</td>
<td>3.16 ± 0.73*</td>
<td>3.78 ± 0.52*†</td>
<td>3.19 ± 0.66*</td>
<td>0.022</td>
</tr>
<tr>
<td>LV S_long, %</td>
<td>-22.7 ± 5.19</td>
<td>-16.0 ± 6.27*</td>
<td>-21.4 ± 1.84*†</td>
<td>-19.0 ± 4.35*†</td>
<td>0.007</td>
</tr>
<tr>
<td>LA strain %</td>
<td>21.5 ± 2.63</td>
<td>13.9 ± 2.38*</td>
<td>16.7 ± 0.64*†</td>
<td>17.0 ± 3.73*†</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are presented as the mean ± SD; n, number of rats; T4, L-thyroxine; Irb, irbesartan; Pro, propranolol; E, peak E velocity; A, peak A velocity; DT, deceleration time; Ea, peak early diastolic mitral annular velocity; Sm, peak systolic mitral annular velocity; LV, left ventricular; S_long, peak longitudinal strain; LA, left atrial. *P < 0.05 vs. control; †P < 0.05 vs. T4 alone; ‡P < 0.05 vs. T4 Pro.

Fig. 3 Mitral and mitral annular flow velocity in control (A, C) and T4 alone rat (B, D). Peak late diastolic velocity was significantly higher in T4 alone rat (B). Peak systolic mitral annulus (Sm) was lower in T4 alone rat compared with control (D).
925RAS in thyrotoxic cardiomyopathy

1.00 μm vs. 25.0 ± 1.77 μm, P < 0.001) compared with T4 alone. There was no significant difference in myocyte width between T4-Pro and T4 alone (24.2 ± 1.30 μm vs. 25.0 ± 1.77 μm, P = 0.79). None of the sections showed apoptosis, necrosis or fat droplets microscopically.

**Interstitial connective tissue**

To evaluate whether TH-induced cardiac hypertrophy was accompanied by cardiac fibrosis, we examined the presence of collagen fibers in the transverse sections. Corresponding Trichrome stains confirmed the presence of fibrosis. Compared with control, all samples of T4 alone displayed marked inflammation and extracellular matrix fibrosis (Fig. 4). T4-Irb showed significantly reduced number of inflammation foci and extent of fibrosis compared with T4 alone. However, there were no significant changes between T4-Pro and T4 alone (Table 4). Cardiomyocyte width and LV S_long in rats with fibrosis (n=9, 24.8 ± 1.79 and -14.4 ± 3.69) were significantly reduced compared with those in rats without fibrosis (n=11, 20.3 ± 1.82 % and -22.9 ± 4.02%, all P < 0.001). There was also a good correlation between changes in LV S_long and presence of fibrosis (r = 0.76, P < 0.001) (Table 5).

**Table 4** Microscopic measurements

<table>
<thead>
<tr>
<th></th>
<th>Cardiomyocyte width (μm)</th>
<th>Fibrosis</th>
<th>No of inflammation foci</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control 2</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control 3</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control 4</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control 5</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>T4 alone group</strong></td>
<td>25.0 ± 1.77*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 alone 1</td>
<td>25</td>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td>T4 alone 2</td>
<td>27.5</td>
<td>Mild</td>
<td>5</td>
</tr>
<tr>
<td>T4 alone 3</td>
<td>22.5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>T4 alone 4</td>
<td>25</td>
<td>Mild</td>
<td>2</td>
</tr>
<tr>
<td>T4 alone 5</td>
<td>25</td>
<td>Mild</td>
<td>3</td>
</tr>
<tr>
<td><strong>T4 Irb group</strong></td>
<td>21.0 ± 1.00+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4, Irb 1</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T4, Irb 2</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T4, Irb 3</td>
<td>22</td>
<td>Mild</td>
<td>1</td>
</tr>
<tr>
<td>T4, Irb 4</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T4, Irb 5</td>
<td>21</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>T4 Pro group</strong></td>
<td>24.2 ± 1.30‡</td>
<td></td>
<td></td>
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<tr>
<td>T4, Pro 1</td>
<td>26</td>
<td>Mild</td>
<td>2</td>
</tr>
<tr>
<td>T4, Pro 2</td>
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<tr>
<td>T4, Pro 3</td>
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<td>Mild</td>
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</tr>
<tr>
<td>T4, Pro 4</td>
<td>25</td>
<td>Mild</td>
<td>4</td>
</tr>
<tr>
<td>T4, Pro 5</td>
<td>23</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*T4, L-thyroxine; Irb, irbesartan; Pro, propranolol; *P < 0.001 vs. control; †P < 0.001 vs. T4 alone; ‡P = 0.79 vs. T4 alone

1.00 μm vs. 25.0 ± 1.77 μm, P < 0.001) compared with T4 alone. There was no significant difference in myocyte width between T4-Pro and T4 alone (24.2 ± 1.30 μm vs. 25.0 ± 1.77 μm, P = 0.79). None of the sections showed apoptosis, necrosis or fat droplets microscopically.

**Fig. 4** Microscopic findings of control myocardium (A) and T4 alone rat (B, C). Myocardial fibers were disarrayed due to fibrosis in T4 treated rat. (H&E stain, x200)(B). Fibrotic area showed blue color on Masson-Trichrome stain (special stain, Masson-Trichrom, x200) (C).
thyroid cardiomyopathy, because most hyperthyroid patients display diastolic dysfunction with conserved LVEF [19]. TH increases total blood volume and cardiac preload, but pacing-induced increase in contraction frequency generally reduces preload and stroke volume and increase of HR reduces diastolic filling time, leading to greater dependence on atrial systole [1]. The finding of unchanged LVEDD and increased indices of E/Ea in our thyrotoxic rats should be considered indicative of an effective increase in preload. Higher peak A velocity with reduction of E/A ratio and Ea velocity, and increase of E/Ea are evidence of the prominent diastolic dysfunction in thyrotoxic rats. Impairment in LV relaxation in thyrotoxic rats may result in reduction of early LA emptying, evidenced by low Ea. This impaired emptying contributes to a larger residual volume before LA contraction. The presence of such volumetric changes and increased LA pressure reduce longitudinal contraction, as suggested by the lower values of peak systolic strain of LA, which may have a potential impact on the development of atrial arrhythmias.

Although early stage hyperthyroid cardiomyopathy usually is associated with normal LVEF, radial shortening does not always guarantee normal LV systolic function [20]. In our study, eight of ten in T4 alone rats showed more deteriorated LV S long despite similar EF and FS compared with control. Since longitudinal shortening is governed by longitudinal subendocardial fibers, which are more vulnerable to myocardial ischemia, assessment of LV longitudinal function based on strain echocardiography may be a sensitive marker for detecting subclinical alterations in LV systolic performance [20, 21]. Analysis with speckle tracking strain imaging measures wall motion velocities relatively irrespective of volume, contrary to EF, and has been validated for the evaluation of longitudinal LV function [21, 22]. Subclinical LV dysfunction relates to the structure-function relationship and can be detected by a decrease in longitudinal myocardial function, which occurs before the development of abnormality in conventional measures of LV performance [23].

**Role of irbesartan and propranolol in TH induced LV hypertrophy**

Proposed causes of impairment in myocardial contraction and relaxation in TH-induced heart failure
include increased HR and afterload, and neurohormonal activation, each of which could induce LV hypertrophy [1]. T4 alone rats in our study also showed the increased HW/BW and cardiac hypertrophy estimated by a slight increase in the ratio of LV wall thickness to LV chamber size and enlargement of cross-sectional myocyte width. In case with chronic hyperthyroidism, which is typically associated with simultaneous increases in BP, pressure overload-induced cardiac hypertrophy could play a role in the development of cardiac hypertrophy. However, in our relatively short-term study, there was no difference in SBP between T4-alone and control rats, indicating that the cardiac hypertrophy was not due to elevated hemodynamic parameters and same result was shown by Hu LW et al. [24]. Moreover, hyperthyroidism is associated with increased cardiac β-receptor density [25], and an increased expression of β1-adrenergic receptor mRNA is apparent in response to thyroxine administration [26]. But, this was associated with decreased expression in pressure overload-induced cardiac hypertrophy, suggesting that the adrenergic system could play a role in the development of cardiac hypertrophy in chronic hyperthyroidism.

In our study, cardiac hypertrophy and the ventricular geometry pattern assessed by echocardiography induced by T4 administration was not significantly changed by the co-administration with propranolol, despite decreased LV filling pressure assessed by E/Ea compared with T4 alone. These data are in agreement with those of Cooper et al. [27], who reported that adrenergic stimulation in cats is not necessary for T4-induced hypertrophy. However, other studies reported that the SNS plays an important role in the development of cardiac hypertrophy [28] and propranolol administration prevents hyperthyroidism-induced cardiac hypertrophy [13]. Together, these results suggest that the effects of T4 are not mediated solely through the adrenergic nervous system and corroborate the findings of Asahi et al. [29] showing that the RAS is an important modulator of T4-induced cardiac hypertrophy.

Recently, some investigators have suggested that the RAS plays an important role in the development of cardiac hypertrophy in thyrotoxic rats, demonstrating an activated circulating RAS in hyperthyroidism [14-17]. In the present study, co-administration with a RAS inhibitor such as irbesartan attenuated cardiac hypertrophy with reduction of cardiomyocyte width and fibrosis in thyrotoxic rats. Although irbesartan significantly reduced baseline SBP after the 4-week treatment, our thyrotoxic rat model is not associated with increase in SBP. These data indicate that factors other than hemodynamic changes play an important role in the pathogenesis of T4-induced cardiac hypertrophy [30, 31], and clinical evidence suggests that blocking the actions of angiotensin II might confer protective benefits beyond lowering BP [32]. Although there is controversy regarding the formation of myocardial fibrosis induced by TH [33-35], considering that RAS is activated in patients with hyperthyroidism [16, 17] and myocardial fibrosis depends on a complex relationship between stimulatory (including angiotensin II, endothelin I, catecholamines, and aldosterone) and inhibitory factors acting on the collagen production [36], it is reasonable to suggest that these mechanisms could mediate the increased myocardial fibrosis observed in our experimental data, suggesting the involvement of TH in regulating myocardial fibrosis. Interestingly, treatment with RAS inhibitor was presently able to prevent myocardial fibrosis. Crawford et al. [37] demonstrated that angiotensin II stimulates fibronectin and expression of collagen types I and IV in association with the proliferation of interstitial fibroblasts by direct action, which could be prevented by the AT1 receptor antagonist losartan. In the present study, altered myocardial function was induced by T4 characterized with reduced LV systolic and diastolic function, and this was correlated with severity of myocardial hypertrophy, inflammation and fibrosis.

In conclusion, our results suggest that irbesartan could significantly attenuate structural and functional remodeling induced by experimental thyrotoxic cardiomyopathy, which is not found with propranolol, although regulation of HR is evident with adrenergic blocker. These results may implicate the RAS as having an important role in thyrotoxic cardiomyopathy beyond tachycardia.

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Disclosure Statement

The authors declare that no competing financial interests exist.
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