Changes in left ventricular function and remodeling after myocardial infarction in hypothyroid rats

Yue-Feng Chen, Rebecca A. Redetzke, Suleman Said, April J. Beyer, and A. Martin Gerdes

Cardiovascular Health Research Center, Sanford Research/University of South Dakota, Sioux Falls, South Dakota

Submitted 12 August 2009; accepted in final form 6 November 2009

HYPOTHYROIDISM IS A common condition in the general population. The prevalence rate is 4.6% in the United States population (0.3% clinical, 4.3% subclinical), is higher in females than in males, and increases with age (4). Hypothyroidism has been found to be associated with an increased risk of cardiovascular diseases such as atherosclerosis and myocardial infarction (MI) (3, 19). Hypothyroidism has also been shown to delay wound healing in experimental MI rats (9) and causes increased MI size in dogs (7). However, the long-term effect of preexisting hypothyroidism on post-MI remodeling is unknown.

In this study, thyroidectomized (TX) rats were used to investigate the influence of hypothyroidism on LV remodeling after MI. We found that hypothyroid rats developed cardiac atrophy and had less LV chamber dilatation but more severe LV dysfunction 4 wk after MI.

MATERIALS AND METHODS

Experimental design. Adult female euthyroid (non-TX) and TX Sprague-Dawley rats weighing between 209 and 257 g were purchased from Charles River Laboratories (Wilmington, MA). MI surgery was performed 4 wk after TX. Animals from non-TX or TX groups were randomly assigned to MI or sham-MI surgery, respectively. MI was produced by a ligation of the left descending coronary artery as described in previous publications; sham MI was produced with a similar procedure, except the ligature was loosely tied (1, 15). Survivors were placed into the following groups: 1) TX + MI (n = 8), 2) TX + sham-MI (n = 10), 3) non-TX + MI (MI, n = 11), and 4) non-TX sham-MI group (S, n = 10). Animals were housed two per cage and kept on a 12-h:12-h light-dark cycle with food and water provided ad libitum. Pre-MI surgery echocardiographic data were collected 4 wk after TX. In terminal experiments 4 wk later, cardiac function was assessed by echocardiography and LV catheterization for each animal in the study. All experiments and protocols were approved by the University of South Dakota Animal Care and Use Committee.

Echocardiographic measurements. Echocardiography was performed in each animal before it was euthanized using a Visualsonics 660 imaging system (20-MHz transducer; Toronto, Canada) as described previously (1, 16). Briefly, rats were anesthetized using isoflurane (1.5%). Two-dimensional echocardiograms were obtained from short-axis views of the LV at the level of the papillary muscle tips. Two-dimensionally targeted M-mode echocardiograms were used to measure the LV dimensions in systole and diastole.

Cardiac hemodynamic measurements. LV hemodynamics were obtained by catheterization of the right carotid artery using a Millar Micro-tip catheter (Millar Instruments; Houston, TX) as described previously (1, 22). Data were recorded using a Digi-Med Heart Performance Analyzer system (model HPA 410a; MicroMed; Louisville, KY). The following data were collected: heart rate, LV peak systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), and positive/negative change in pressure over time (∆P/dt).

Tissue collection. After hemodynamic data were collected, the chest was opened and the hearts were arrested in diastole by an intravenous injection of saturated potassium chloride solution via inferior vena cava, quickly removed, and immediately placed in ice-cold PBS. The hearts were cannulated with an 18-gauge gavage needle through the aorta, perfused with ice-cold PBS, and subsequently trimmed, blotted, and weighed. The LV plus septum and the right ventricle (RV) were dissected and weighed. The LV was then cut into three pieces transversely, perpendicular to the LV long axis. The middle slice, which was cut 1 mm below the suture, was fixed in 4% paraformaldehyde overnight, then transferred to PBS, embedded in optimum cutting temperature compound (Sakura Finetek; Torrance, CA), and cryosectioned at 5 μm thickness for histology.

Infarct size measurement. Paraformaldehyde-fixed transverse tissue sections were obtained by cryosection, stained with hematoxylin and eosin, and viewed under a microscope with a color video camera to identify infarcted and noninfarcted areas and the border between these areas. Infarct length = (infarcted tissue outer length + infarcted tissue inner length)/2. Infarct size was estimated by measuring the percentage of endocardial and epicardial circumferences replaced by infarcted tissue using the following formula: infarct size (in %) = [(infarcted tissue outer length + infarcted tissue inner length)/(LV transversal epicardial circumference + LV transversal endocardial circumference)] × 100% (10, 14). MI animals with small infarct size.
H260

HYPOTHYROIDISM EXACERBATES POST-MI REMODELING

Table 1. Serum thyroid hormone levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>TSH, μIU/ml</th>
<th>Total T3, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-TX sham</td>
<td>10</td>
<td>0.53 (SD 0.48)</td>
<td>1.25 (SD 0.03)</td>
</tr>
<tr>
<td>TX</td>
<td>18</td>
<td>10.85 (SD 9.67)*</td>
<td>0.43 (SD 0.18)*</td>
</tr>
</tbody>
</table>

Values are means (SD); n, number of animals. TSH, thyroid-stimulating hormone; T3, triiodothyronine; TX, thyroidectomized. *P < 0.01 vs. non-TX sham (2-tailed Student’s t-test).

Table 2. Body weight and heart weight data

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Body Wt1, g</th>
<th>Body Wt2, g</th>
<th>Heart Wt, mg</th>
<th>HW/BW2</th>
<th>Ventricular Wt, mg</th>
<th>LV Wt, mg</th>
<th>RV Wt, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-TX</td>
<td>10</td>
<td>229 (SD 5)</td>
<td>251 (SD 12)</td>
<td>872 (SD 79)</td>
<td>3.5 (SD 0.3)</td>
<td>772 (SD 66)</td>
<td>625 (SD 53)</td>
<td>147 (SD 18)</td>
</tr>
<tr>
<td>TX</td>
<td>11</td>
<td>229 (SD 6)</td>
<td>253 (SD 10)</td>
<td>1,040 (SD 132)†</td>
<td>4.1 (SD 0.6)*</td>
<td>863 (SD 100)*</td>
<td>655 (SD 64)</td>
<td>208 (SD 62)*</td>
</tr>
<tr>
<td>%Difference</td>
<td>0</td>
<td>1</td>
<td>19</td>
<td>17</td>
<td>17</td>
<td>12</td>
<td>5</td>
<td>41</td>
</tr>
</tbody>
</table>

Table 3. Infarct size

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Infarct Length, mm</th>
<th>Infarct Size, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-TX MI</td>
<td>11</td>
<td>14.3 (SD 2.1)</td>
<td>41.3 (SD 5.0)</td>
</tr>
<tr>
<td>TX MI</td>
<td>8</td>
<td>16.7 (SD 3.3)</td>
<td>53.6 (SD 9.2)*</td>
</tr>
</tbody>
</table>

Values are means (SD); n, number of animals. *P < 0.05 vs. non-TX MI (2-tailed Student’s t-test).

RESULTS

Serum thyroid hormone levels. Table 1 shows the serum TSH and total T3 levels in terminal experiments. TX rats (with or without MI) had significantly increased TSH and decreased total T3 levels compared with non-TX sham-MI rats, confirming the success of TX surgery. Although the serum TSH levels showed some variability, all TX rats had higher TSH levels and lower total T3 levels than non-TX rats. There was no difference in thyroid levels between TX sham-MI and TX MI rats, so the data were pooled for statistical analysis.

Changes in body weight and heart weight. Table 2 shows that 4 wk after TX surgery, there were no significant differences in body weight between TX and non-TX rats, but TX MI rats showed less post-MI weight gain. In non-TX animals, 4 wk MI led to increased heart weight, heart weight-to-body weight ratio, and ventricular weight (particularly RV weight), suggesting LV dysfunction. TX animals from both MI and sham-operated groups had significantly smaller heart weights and ventricular weights than non-TX animals. After 4 wk, MI tended to increase heart weight and ventricular weight in TX animals, but this did not reach statistical significance compared with TX sham-MI animals, and the hypertrophic response was attenuated compared with that in non-TX MI rats. Although infarct length was 17% larger in TX-MI rats compared with non-TX MI rats, this did not reach statistical significance (P = 0.16) (Table 3). However, when compared with non-TX MI rats, TX MI rats had a significantly larger increase in percent infarct size because of their smaller hearts.

Echocardiographic changes. Four weeks after TX surgery, TX rats had smaller hearts with impaired LV function compared with age-matched non-TX rats. TX rats were characterized by smaller LV end-diastolic chamber dimensions but larger LV end-systolic chamber dimensions, thinner walls, and reduced heart rate, with decreased fractional shortening (Table 4). Table 5 shows that MI led to increased chamber dimensions and reduced fractional shortening in both TX and non-TX groups. In general, percent changes in these parameters tended to be less in TX rats with preexisting adverse remodeling before the infarction.

Hemodynamic changes after MI. Hemodynamic changes are shown in Table 6. Eight weeks after surgical TX, TX rats had decreased LVSP and decreased ±dP/dt compared with non-TX rats. In non-TX rats, MI led to an increase in LVEDP and decreases in LVSP, +dP/dt, and −dP/dt. In TX rats, MI led to a more pronounced increase in LVEDP and decrease in −dP/dt (e.g., greater percent change than in non-TX rats).

DISCUSSION

This study shows that 4 wk of hypothyroidism led to reduced heart size, decreased LV chamber dimension and wall thickness, and impaired LV systolic and diastolic function as indicated by decreased fractional shortening and ±dP/dt. This is consistent with previous reports (17, 20). However, chamber dilatation was not yet observed 4 wk after TX as was noted after 6 wk propylthiouracil treatment in a previous study from our laboratory (17). This may be related to the extent of

Additional references and source information are provided in the footnotes and references section of the original article.
HYPOTHYROIDISM EXACERBATES POST-MI REMODELING

Table 4. Pre-MI surgery echocardiographic data

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>HR, beats/min</th>
<th>IVSd, mm</th>
<th>IVSs, mm</th>
<th>LVIDd, mm</th>
<th>LVIDs, mm</th>
<th>LVPWTd, mm</th>
<th>LVPWTs, mm</th>
<th>FS, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-TX</td>
<td>6</td>
<td>372 (SD 47)</td>
<td>1.5 (SD 0.4)</td>
<td>2.6 (SD 0.2)</td>
<td>7.4 (SD 0.4)</td>
<td>4.0 (SD 0.9)</td>
<td>1.6 (SD 0.3)</td>
<td>2.8 (SD 0.5)</td>
<td>46 (SD 11)</td>
</tr>
<tr>
<td>TX</td>
<td>12</td>
<td>266 (SD 25)†</td>
<td>1.2 (SD 0.4)</td>
<td>1.8 (SD 0.5)†</td>
<td>6.5 (SD 0.5)†</td>
<td>4.8 (SD 0.6)*</td>
<td>1.2 (SD 0.2)*</td>
<td>1.6 (SD 0.3)†</td>
<td>27 (SD 6)†</td>
</tr>
</tbody>
</table>

Values are means (SD); n, number of animals. HR, heart rate; IVSd and IVSs, interventricular septal thickness in end diastole and systole, respectively; LVIDd and LVIDs, left ventricular diastolic and systolic internal diameter, respectively; LVPWTd and LVPWTs, left ventricular diastolic and systolic posterior wall thickness, respectively; FS, fractional shortening. *P < 0.05 and †P < 0.01 vs. non-TX (2-tailed Student’s t-test).

Table 5. Post-MI surgery echocardiographic data

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>HR, beats/min</th>
<th>IVSd, mm</th>
<th>IVSs, mm</th>
<th>LVIDd, mm</th>
<th>LVIDs, mm</th>
<th>LVPWTd, mm</th>
<th>LVPWTs, mm</th>
<th>FS, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-TX</td>
<td>10</td>
<td>311 (SD 36)</td>
<td>1.8 (SD 0.3)</td>
<td>2.9 (SD 0.3)</td>
<td>7.2 (SD 0.4)</td>
<td>4.0 (SD 0.4)</td>
<td>1.7 (SD 0.8)</td>
<td>2.9 (SD 1.1)</td>
<td>45 (SD 5.2)</td>
</tr>
<tr>
<td>MI</td>
<td>11</td>
<td>337 (SD 49)</td>
<td>1.7 (SD 0.2)</td>
<td>2.3 (SD 0.8)</td>
<td>9.8 (SD 0.8)b</td>
<td>8.0 (SD 1.4)b</td>
<td>1.5 (SD 0.4)</td>
<td>1.9 (SD 0.5)</td>
<td>19 (SD 8.6)b</td>
</tr>
<tr>
<td>%Difference</td>
<td>8</td>
<td>–6</td>
<td>–21</td>
<td>36</td>
<td>100</td>
<td>–12</td>
<td>–34</td>
<td>–58</td>
<td></td>
</tr>
<tr>
<td>TX</td>
<td>10</td>
<td>244 (SD 32)b,d</td>
<td>1.4 (SD 0.3)b</td>
<td>2.2 (SD 0.3)b</td>
<td>6.2 (SD 0.6)b,d</td>
<td>4.1 (SD 0.4)d</td>
<td>1.5 (SD 0.4)</td>
<td>2.0 (SD 0.4)</td>
<td>33.8 (SD 5.6)b,d</td>
</tr>
<tr>
<td>MI</td>
<td>7</td>
<td>254 (SD 38)b,e</td>
<td>1.4 (SD 0.2)e</td>
<td>2.1 (SD 0.3)e</td>
<td>7.4 (SD 0.9)e</td>
<td>5.7 (SD 1.2)e</td>
<td>1.6 (SD 0.4)</td>
<td>2.0 (SD 0.5)</td>
<td>23.6 (SD 8.2)b,e</td>
</tr>
<tr>
<td>%Difference</td>
<td>4</td>
<td>0</td>
<td>–5</td>
<td>19</td>
<td>39</td>
<td>7</td>
<td>0</td>
<td>–30</td>
<td></td>
</tr>
</tbody>
</table>

Values are means (SD); n, number of animals. *P < 0.05 and bP < 0.01 vs. non-TX sham; †P < 0.05 and †P < 0.01 vs. non-TX MI; ‡P < 0.01 vs. TX sham (ANOVA with Student-Newman-Keuls multiple comparison test between groups).

hypothyroidism induced and/or the earlier observation time point in the current study. We have previously reported that the reduction of heart size in hypothyroidism of 4 wk duration was caused by myocyte atrophy, mainly by reduced myocyte cross-sectional area that was responsible for changes in wall thickness (12).

In response to MI, euthyroid rats showed increased heart weight, especially RV weight, with LV chamber dilatation; increased LVEDP; and decreased ±dP/dt. It appears that TX had an adverse effect on the infarct scar as evidenced by a tendency for increased infarct length and a significant increase in percent infarct size. There was a lower hypertrophic response in TX rats after MI as evidenced by an attenuation of the increases in heart weight (8% vs. 19% in euthyroid rats) and LV diastolic chamber dimension (19% vs. 36% in euthyroid rats). Importantly, there was a more pronounced increase in LVEDP (206% vs. 93% in non-TX rats) and decline in ±dP/dt (45% vs. 27% in non-TX rats) in TX rats after MI. These data suggest that hypothyroidism may exacerbate post-MI LV remodeling with further impairment of LV function, particularly diastolic function. These changes may be of particular significance since increased diastolic dysfunction post-MI has been shown to predict a worse outcome (8, 13).

In euthyroid rats 4 wk after MI, the LV chamber dimension increased and the wall thickness tended to decrease, suggesting a volume overload effect due to the loss of cardiac muscle mass. In TX rats, MI also caused an increased LV chamber dimension but with a preserved wall thickness, although these rats had a thinner wall thickness to begin with. We cannot exclude the possibility of coexisting cardiac myxema and interstitial collagen deposition as reports have shown that hypothyroidism can cause cardiac myxema (18) and is associated with an increased collagen deposition in the heart (21). The increased LV dimension-to-wall thickness ratio in MI rats will lead to an increase in LV wall stress and stretch, resulting in an increase in LV diastolic pressure. However, as the LV in MI rat is nonspherical, with varying wall thickness, anisotropic and nonhomogeneous material properties, the use of the law of Laplace to predict LV pressure-volume and pressure-wall stress behavior is limited. A finite element analysis has been used by others in larger models (5, 6, 11) but is beyond the scope of the present study.

In this study, infarct size was measured 4 wk after MI, when scar formation was complete. Increased percent infarct size was observed in hypothyroid rats. This is consistent with a study by Karlsberg et al. (7) where it was reported that hypothyroidism caused increased infarct size in dogs after acute MI. In that study, increased infarct size was suggested by increased serum creatine kinase levels rather than anatomical measurements as done here. The cause of infarct size increase is unclear but may relate to the reduction in arteriolar density present in hypothyroidism as reported previously by our group (17). This anatomical impairment in vascular density and the associated state of chronic vasoconstriction in hypothyroidism may adversely affect myocyte survival, particularly in the border zone. Other factors such as mitochondrial loss may also contribute (7).

The presence of preexisting hypothyroidism followed by MI has been documented rarely in the clinical setting. Comtois et al. (2) reported a 0.3% (17 of 5,695 patients) incidence of this condition at their institution. They also reported a trend for higher creatine kinase peak levels and an increased incidence of residual ischemia in these patients. However, other methods were used to measure the infarct size in their study. In addition, there was no increase in residual heart failure and mortality rate in these patients. Unlike these patients, hypothyroid MI rats in the current study showed further impairment of LV function, possibly related to the presence of preexisting LV dysfunction from hypothyroidism. The greater increase in LVEDP in hypothyroid MI rats may be due to increased wall stiffness, since hypothyroid-induced cardiac myxema (18) and collagen deposition (21) may worsen after MI. Unlike these rats, which have profound hypothyroidism after thyroidectomy, subclinical hypothyroidism is more common in the general population, especially in the elderly. It would be important to determine
whether the findings from the present study are present in milder forms of low thyroid function.

Conclusion. Our study demonstrates that preexisting hypothyroidism may exacerbate post-MI LV remodeling with further impairment of LV function, particularly diastolic function.

DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


