Effects of Statin Therapy on the Production of Monocyte Pro-Inflammatory Cytokines, Cardiac Function, and Long-Term Prognosis in Chronic Heart Failure Patients With Dyslipidemia

Akihiro Nakagomi, MD; Yoshihiko Seino, MD; Keiichi Kohashi, MD; Munenori Kosugi, MD; Yasumi Endoh, MD; Yoshiki Kusama, MD; Hirotugu Atarashi, MD; Kyoichi Mizuno, MD

Background: The effects of statin therapy on the production of monocyte pro-inflammatory cytokines, cardiac function and the long-term prognosis in chronic heart failure (CHF) patients with dyslipidemia remain unclear.

Methods and Results: A total of 146 CHF patients with a mean left ventricular ejection fraction (LVEF) of 26.9±6.6% were divided into 2 groups based on whether or not statins were included in their treatment: a statin group (n=63) and a no statin group (n=83). Only patients with dyslipidemia were treated with statins. Peripheral blood mononuclear cells (PBMCs) were isolated, and the production of monocyte tumor necrosis factor (TNF)-α and interleukin (IL)-6 were measured at baseline and after 6 months of treatment, and the data expressed as mean±SD (pg·mL⁻¹·10⁻⁶ PBMCs). The LVEF in the statin group improved, and the monocyte TNF-α and IL-6 production decreased (respectively, P<0.001), but the LVEF and cytokine production remained unchanged in the no statin group. Multivariate Cox hazard analysis showed that statin therapy (hazard ratio, 0.14; 95% confidence interval: 0.02–0.97, P=0.046) was an independent predictor of cardiac events.

Conclusions: Statin therapy attenuates the production of monocyte pro-inflammatory cytokines, and ameliorates the cardiac function and may improve long-term prognosis in CHF patients with dyslipidemia. (Circ J 2012; 76: 2130–2138)

Key Words: Cytokine; Dyslipidemia; Heart failure; Statin
Subjects
The present study was a prospective study and included 146 selected patients with CHF (106 male and 40 female; mean age, 65.3±11.1 years) with a mean left ventricular ejection fraction (LVEF) of 26.9±6.6%. A total of 45 age- and gender-matched normal subjects (29 male and 16 female; mean age, 64.7±10.7 years) were included for comparison. The etiology of CHF was DCM in 114 patients and ischemic cardiomyopathy (ICM) in 32 patients. DCM was defined as a normal coronary arteriogram together with severe hypokinesis of the left ventricular wall motion as determined on left ventriculography and from the typical pathological findings of an endomyocardial biopsy of the left ventricle. ICM was defined as a history of myocardial infarction with significant coronary artery disease (>70% luminal stenosis in at least 2 major coronary arteries). LVEF was measured on echocardiography within 1 week of the measurement of the biochemical markers. All echocardiograms of the eligible patients were analyzed by 2 blinded trained cardiologists. All eligible patients had been receiving standard medical treatment for at least 3 months prior to enrollment in the present study. In addition, patients had received β-blockers or spironolactone for at least 1 year before enrollment.

The patients were considered to have dyslipidemia if they had an overnight fasting serum total cholesterol (TC) ≥220 mg/dl, triglycerides (TG) ≥150 mg/dl, LDL-C ≥140 mg/dl, or high-density lipoprotein-cholesterol (HDL-C) ≤40 mg/dl. LDL-C level was calculated using the Friedwald formula. Patients were divided into 2 groups based on whether or not statins were included in their treatment: a statin group (n=63) and a no statin group (n=83). Only patients with dyslipidemia were treated with statins. None of the patients had received any lipid-modulating medications, including statins or fibrates, before enrollment.

Follow-up and Determination of Outcomes
All patients were followed up for a median of 60.0 months (range; 8–158 months) to determine the incidence of cardiac events such as cardiac death and readmission due to a worsening of CHF.

Reagents and Laboratory Measurements
Hanks’ balanced salt solution was obtained from Sigma (Tokyo, Japan) and RPMI 1640 was sourced from GIBCO (Tokyo, Japan). Endotoxin-tested Lymphoprep was obtained from Nycomed Pharma AS (Oslo, Norway). Media, including RPMI 1640 was sourced from GIBCO (Tokyo, Japan). Endotoxin-tested Lymphoprep was obtained from Nycomed Pharma AS (Oslo, Norway). Media, including RPMI 1640 was sourced from GIBCO (Tokyo, Japan). Hanks’ balanced salt solution was obtained from Sigma (Tokyo, Japan) and RPMI 1640 was sourced from GIBCO (Tokyo, Japan). Media, including RPMI 1640 was sourced from GIBCO (Tokyo, Japan).

C-reactive protein (CRP) level was measured on immunoturbidimetry assay. Plasma B-type natriuretic peptide (BNP) concentrations were determined with a specific immunoradiometric assay for human BNP using commercial kits (Shionoria Kit, Shionogi and Kyowa Medex, Tokyo, Japan). The performance characteristics of the Shionoria BNP kit are: coefficient of variance, 2.5–4.3% (n=10); analytical range, 4–2,000 pg/ml. To determine renal function, the estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease prediction equation for Japanese subjects (194 x serum creatinine^−1.004 [mg/dl] x Age^−0.287 [years] x 0.739, if female). 9

Statistical Analysis
The results are presented as mean±SD for continuous variables and as the percentage of the total number of patients for categorical variables. Student’s t-test for independent samples and the chi-square test were used for comparisons of the continuous and categorical variables, respectively. Cytokine level and CRP were skewed distributions. Therefore, the Mann-Whitney test was used for unpaired comparisons between the groups, and Wilcoxon’s signed rank test was used for paired comparisons within the groups. To compare the production of monocyte cytokines among the 3 groups, the Kruskal-Wallis test was used for unpaired comparisons between the groups, and Wilcoxon’s signed rank test was used for paired comparisons within the groups. The bivariate correlations between parameters were assessed with the Pearson or Spearman correlation (r) coefficient.
Table 1. Baseline Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects (n=45)</th>
<th>All CHF patients (n=146)</th>
<th>P value [Normal vs. CHF]</th>
<th>CHF patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.7±11.3</td>
<td>65.3±11.1</td>
<td>0.740</td>
<td>64.3±9.9</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>29 (64.4)</td>
<td>106 (72.6)</td>
<td>0.349</td>
<td>44 (69.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.3±1.3</td>
<td>22.0±2.2</td>
<td>0.199</td>
<td>22.7±2.4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117±7</td>
<td>118±8</td>
<td>0.760</td>
<td>119±8</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>75±4</td>
<td>76±4</td>
<td>0.295</td>
<td>76±4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>70±6</td>
<td>75±7</td>
<td>&lt;0.001</td>
<td>75±8</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.3±0.7</td>
<td>12.3±1.3</td>
<td>&lt;0.001</td>
<td>12.4±1.3</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.11±0.04</td>
<td>0.45±0.18</td>
<td>&lt;0.001</td>
<td>0.43±0.16</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>189±15</td>
<td>204±28</td>
<td>0.001</td>
<td>228±18</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>110±13</td>
<td>130±23</td>
<td>&lt;0.001</td>
<td>151±16</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>60±6</td>
<td>46±6</td>
<td>&lt;0.001</td>
<td>45±7</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>101±21</td>
<td>142±35</td>
<td>&lt;0.001</td>
<td>165±37</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>97±7</td>
<td>106±16</td>
<td>&lt;0.001</td>
<td>108±15</td>
</tr>
<tr>
<td>eGFR (ml · min⁻¹ · 1.73m⁻²)</td>
<td>64.1±8.8</td>
<td>46.7±10.5</td>
<td>&lt;0.001</td>
<td>46.6±11.0</td>
</tr>
</tbody>
</table>

Data given as mean±SD or n (%).

CHF, chronic heart failure; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; Hb, hemoglobin; CRP, C-reactive protein; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; TG, triglycerides; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate.

Results

Subjects

The baseline clinical characteristics of the normal subjects and patients with CHF are shown in Table 1. Patients with CHF before statin therapy had a significantly higher heart rate, serum TC, LDL-C, TG, fasting plasma glucose (FPG), and CRP level, and had lower Hb, HDL-C, and eGFR in comparison to normal subjects.

The baseline clinical characteristics of patients receiving statins (statin group) and those not receiving statins (no statin group) are also given in Table 1. The BMI, TC, LDL-C and TG levels before statin therapy were significantly higher in the statin group than in the no statin group (Table 1). The etiology of CHF and the NYHA class were similar between the statin and no statin groups (DCM/ICM, 47/16 vs. 67/21, P=0.422; NYHA class II/III/IV, 39/23/1 vs. 48/30/5, P=0.403).

Changes in Clinical Characteristics According to Statin Treatment

The percent changes in the clinical characteristics from baseline to 6 months in patients according to statin treatment are shown in Table 2. After 6 months of treatment, BNP, CRP, TC, LDL-C and TG in the statin group were all significantly decreased, by 34.2%, 16.6%, 17.5%, 23.0%, and 14.8%, respectively. At 6 months, the BMI was significantly decreased by 1.2% in the no statin group (P=0.001), but it was unchanged in the statin group (P=0.835). The BMI in patients treated with statins was significantly higher than that in those treated without statins at 6 months (P=0.001). TC, LDL-C and HDL-C were similar between the groups at 6 months, but TG was still higher in the statin group than in the no statin group (136±11 mg/dl vs. 123±22 mg/dl, P<0.001). In addition, patients in the statin group had significantly lower BNP and CRP in comparison to those in the no statin group (BNP, 290.8±226.4 pg/ml vs. 543.5±394.7 pg/ml, P<0.001; CRP, 0.35±0.16 mg/dl vs. 0.43±0.18 mg/dl, P<0.001).

Changes in LVEF According to Statin Treatment

The changes in the LVEF from baseline to 6 months after initiating treatment are shown in Table 2, Figure 1. LVEF was significantly improved after 6 months of treatment in the statin group (from 27.9±6.8% to 34.3±10.5%, P<0.001), but the LVEF remained unchanged in the no statin group (from 26.1±6.3% to 26.8±8.0%, P=0.194). Furthermore, the LVEF at 6 months was significantly higher in the statin group than in the no statin group (P<0.001).

Effect of CHF on Monocyte Cytokine Production

At baseline, the production of monocyte TNF-α and IL-6 was significantly higher in patients with CHF than in normal subjects (TNF-α, 4.0±1.7 pg · ml⁻¹ vs. 1.9±0.6 pg · ml⁻¹, P<0.001; IL-6, 142.2±97.1 pg · ml⁻¹ vs. 10.6 PBMCs, P<0.001; IL-6, 142.2±97.1 pg · ml⁻¹ vs. 10.6 PBMCs, P<0.001).
Effects of Statins on Heart Failure

The production of monocyte cytokines was significantly and negatively related to the LVEF (TNF-α, r=-0.640, P<0.001; IL-6, r=-0.621, P<0.001). These data suggest that the upregulation of monocyte cytokine production may be, at least in part, due to the increased plasma levels of CRP.

**Effect of Cardiac Events on Monocyte Cytokine Production**

Fifty-four patients experienced cardiac events (23 cardiac deaths; 31 readmissions for worsening CHF), with a median follow-up period of 60.0 months (range, 8–158 months). Of the 23

### Table 2. Change in Clinical Characteristics According to Statin Treatment

<table>
<thead>
<tr>
<th>Statin (n=63)</th>
<th>No statin (n=83)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>At 6 months</strong></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>22.7±2.4</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>119±8</td>
</tr>
<tr>
<td><strong>HR (beats/min)</strong></td>
<td>75±8</td>
</tr>
<tr>
<td><strong>NYHA class (I/II/III/IV)</strong></td>
<td>0/39/23/1</td>
</tr>
<tr>
<td><strong>LVEF (%)</strong></td>
<td>27.9±6.8</td>
</tr>
<tr>
<td><strong>BNP (pg/ml)</strong></td>
<td>466.2±291.6</td>
</tr>
<tr>
<td><strong>CRP (mg/dl)</strong></td>
<td>0.43±0.16</td>
</tr>
<tr>
<td><strong>TC (mg/dl)</strong></td>
<td>228±18</td>
</tr>
<tr>
<td><strong>LDL-C (mg/dl)</strong></td>
<td>151±16</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dl)</strong></td>
<td>45±7</td>
</tr>
<tr>
<td><strong>TG (mg/dl)</strong></td>
<td>165±37</td>
</tr>
<tr>
<td><strong>TNF-α (pg·ml⁻¹·10⁻⁶ PBMCs)</strong></td>
<td>3.9±1.6</td>
</tr>
<tr>
<td><strong>IL-6 (pg·ml⁻¹·10⁻⁶ PBMCs)</strong></td>
<td>135.3±100.3</td>
</tr>
</tbody>
</table>

Data given as mean±SD. *P<0.001 vs. baseline; †P<0.001 vs. patients in the No statin group at 6 months; ‡P=0.017 vs. patients in the No statin group at 6 months.

NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; BNP, B-type natriuretic peptide; TNF, tumor necrosis factor; PBMC, peripheral blood mononuclear cell; IL, interleukin. Other abbreviations as in Table 1.

**Figure 1.** Change in left ventricular ejection fraction (LVEF) from baseline to 6 months of treatment in patients treated with and without statins. LVEF was significantly improved in patients treated with statins after 6 months of treatment (P<0.001), but remained unchanged in patients treated without statins after 6 months (P=0.194).
patients who experienced cardiac death, all patients died from worsening of CHF due to severe pump failure, and none of the patients developed sudden cardiac death or myocardial infarction during the follow-up period.

A comparison of the clinical characteristics according to cardiac events is given in Table 3. The patients with cardiac events were significantly older, and had lower BMI, systolic blood pressure (SBP), LVEF, Hb, eGFR, serum TC, LDL-C and TG, and had a more severe NYHA class in comparison to those without events. Furthermore, the patients with cardiac events had higher heart rate, and higher plasma CRP, BNP and FPG than those without such events.

The production of monocyte TNF-α and IL-6 was significantly higher in patients with cardiac events than in those without events (TNF-α, 5.7±1.1 vs. 3.0±1.0 pg·ml⁻¹·10⁻⁶ PBMCs, P<0.001; IL-6, 215.7±95.4 vs. 99.1±68.2 pg·ml⁻¹·10⁻⁶ PBMCs, P<0.001). In addition, the production of monocyte cytokines in CHF patients without cardiac events was also significantly higher than that in normal subjects (TNF-α, 3.0±1.0 vs. 1.9±0.6 pg·ml⁻¹·10⁻⁶ PBMCs, P<0.001; IL-6, 99.1±68.2 vs. 29.6±24.3 pg·ml⁻¹·10⁻⁶ PBMCs, P<0.001).

**ROC Curve Analysis**

According to ROC curve analysis, the best cut-offs for predicting cardiac events for production of monocyte TNF-α (Figure 2A), LVEF (Figure 2B) and BMI (Figure 2C) were 4.1 pg·ml⁻¹·10⁻⁶ PBMCs, 26%, and 21.0kg/m², respectively. The sensitivity and specificity for production of monocyte TNF-α, LVEF and BMI to predict cardiac events were assessed across a range of cut-offs. The best cut-off for each parameter was associated with a sensitivity of 87.0% (Figure 2A), 80.4% (Figure 2B), and 88.0% (Figure 2C), and specificity of 90.2% (Figure 2A), 70.3% (Figure 2B), and 70.4% (Figure 2C), respectively.

**Monocyte Cytokine Production and LVEF After 6 Months of Treatment**

At baseline, the production of monocyte TNF-α and IL-6 was comparable between patients in the statin group and those in the no statin group (TNF-α, 3.9±1.6 vs. 4.1±1.7 pg·ml⁻¹·10⁻⁶ PBMCs, P=0.488; IL-6, 135.3±100.3 vs. 147.5±94.9 pg·ml⁻¹·10⁻⁶ PBMCs, P=0.460). After 6 months of treatment, the production of monocyte cytokines was significantly decreased (TNF-α, from 3.9±1.6 to 2.8±1.6 pg·ml⁻¹·10⁻⁶ PBMCs, P=0.001; IL-6, from 135.3±100.3 to 96.5±95.8 pg·ml⁻¹·10⁻⁶ PBMCs, P=0.001) in the statin group (Table 2), whereas the production of monocyte cytokines remained unchanged in the no statin group (TNF-α, from 4.1±1.7 to 4.3±2.1 pg·ml⁻¹·10⁻⁶ PBMCs, P=0.553; IL-6, from 147.5±94.9 to 153.9±110.9 pg·ml⁻¹·10⁻⁶ PBMCs, P=0.067; Table 2). In addition, the percent change in the production of TNF-α and IL-6 in the statin group was significantly higher than in the no statin group (Table 2).

The percent change in LVEF from baseline was significantly and inversely correlated with the change in the production of monocyte cytokines (TNF-α, r=−0.460, P<0.001; IL-6, r=−0.637, P<0.001) in the statin group. Interestingly, the percent change in the SBP and diastolic blood pressure did not relate to the change in LVEF after 6 months of treatment (data not shown). These findings suggest that the improvement of cardiac function may have been, at least in part, due to the reduction of monocyte pro-inflammatory cytokines in the statin group.

**Effect of Medications in CHF vs. Presence of Cardiac Events**

The patients in the statin group were less likely to receive angiotensin-converting enzyme inhibitors (ACEIs) and more likely to receive angiotensin receptor blockers (ARBs) in comparison to those without events. The use of furosemide, β-blockers and spironolactone, however, was similar between the groups.

Patients with cardiac events were less likely to receive statins (24.4% vs. 54.3%, P<0.001), β-blockers (75.9% vs. 94.6%, P=0.001) and spironolactone (48.1% vs. 70.7%, P=0.007) in comparison to those without events. The use of ACEIs (70.4% vs. 70.7%, P=0.971), ARBs (31.5% vs. 31.5%, P=0.996) and furosemide (83.3% vs. 88.0%, P=0.158), however, was similar between the groups.

---

**Table 3. Clinical Characteristics vs. Presence of Cardiac Events**

<table>
<thead>
<tr>
<th></th>
<th>Event (+) (n=54)</th>
<th>Event (-) (n=92)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68.6±9.4</td>
<td>63.4±11.6</td>
<td>0.007</td>
</tr>
<tr>
<td>Gender (male, %)</td>
<td>36 (66.7)</td>
<td>70 (76.1)</td>
<td>0.218</td>
</tr>
<tr>
<td>DCM/ICM</td>
<td>39/15</td>
<td>75/17</td>
<td>0.190</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.4±1.7</td>
<td>23.9±1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>116±8</td>
<td>119±8</td>
<td>0.034</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76±4</td>
<td>76±4</td>
<td>0.736</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>78±7</td>
<td>74±7</td>
<td>0.002</td>
</tr>
<tr>
<td>NYHA class (II/III/IV)</td>
<td>12/36/6</td>
<td>75/17/0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.7±1.2</td>
<td>12.7±1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>765.0±279.1</td>
<td>330.5±201.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.62±0.13</td>
<td>0.35±0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>186±26</td>
<td>215±22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>116±20</td>
<td>139±20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>45±6</td>
<td>46±6</td>
<td>0.272</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>124±33</td>
<td>152±32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>110±17</td>
<td>104±15</td>
<td>0.034</td>
</tr>
<tr>
<td>eGFR (ml·min⁻¹·1.73m⁻²)</td>
<td>43.3±7.8</td>
<td>48.6±11.4</td>
<td>0.003</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>21.9±4.4</td>
<td>29.8±5.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy. Other abbreviations as in Tables 1,2.
During a mean follow-up period of 60.0 months (range, 8– 158 months), cardiac events, including 18 cardiac deaths and 23 readmissions due to worsening CHF, occurred in 41 of the 83 patients (49.4%) who did not receive statins, whereas such events (5 cardiac deaths and 8 readmissions for worsening CHF) were diagnosed in only 13 of the 63 patients (20.6%) who received statins (log-rank 10.108, P=0.001; Figure 3A).

In the subgroup analysis, after matching them for use of β-blockers, patients who did not receive statins experienced 29 cardiac events (29/71, 40.8%; 13 cardiac deaths and 16 readmissions for worsening CHF), in comparison to only 12 patients (12/57, 21.1%; 4 cardiac deaths and 8 readmissions due to worsening CHF) who received statins (HR, 0.52; 95% CI: 0.27–0.99, P=0.050). The percent change in LVEF after 6 months was significantly associated with percent change in the production of TNF-α and IL-6 (TNF-α, r=–0.535, P<0.001; IL-6, r=–0.529, P<0.001) even after matching them for use of β-blockers. In addition, after matching them for use of both β-blockers and spironolactone, patients who did not receive statins experienced 17 cardiac events (17/42, 40.5%; 8 cardiac deaths and 9 readmissions for worsening CHF), whereas only 7 patients (7/38, 18.4%; 3 cardiac deaths and 4 readmissions due to worsening CHF) who received statins experienced events (HR, 0.45; 95% CI: 0.24–0.98, P=0.049). The percent change in LVEF after 6 months was significantly related to the percent change in production TNF-α and IL-6 (TNF-α, r=–0.535, P<0.001; IL-6, r=–0.529, P<0.001) after matching them for use of both β-blockers and spironolactone.

**Monocyte TNF-α and IL-6 Production, Serum Lipid Levels, and Cardiac Events**

During a median follow-up period of 60.0 months (range, 8– 158 months), cardiac events including 21 cardiac deaths and 30 readmissions due to worsening CHF occurred in 51 of the 60 patients (85.0%) with elevated TNF-α production (TNF-α ≥4.1 pg · ml⁻¹ · 10⁻⁶ PBMCs, 85.0%), whereas such events (2 cardiac deaths and 1 readmission for worsening CHF) took place in only 3 of the 86 patients (3.5%) without elevated TNF-α production (<4.1 pg · ml⁻¹ · 10⁻⁶ PBMCs, 3.5%; log-rank 87.909, P<0.001; Figure 3B). In addition, cardiac events occurred in 40 of the 59 patients (67.8%) with elevated IL-6 production (IL-6 ≥160 pg · ml⁻¹ · 10⁻⁶ PBMCs, 67.8%), and in contrast, events took place in only 14 of the 87 patients (16.1%) without elevated IL-6 production (<160 pg · ml⁻¹ · 10⁻⁶ PBMCs, 16.1%; log-rank 21.534, P<0.001).

Furthermore, patients with low serum TC (<190 mg/dl) as
NAKAGOMI A et al.

Circulation Journal Vol.76, September 2012

2136

NAKAGOMI A et al.

determined on ROC curve analysis experienced 37 cardiac events (67.3%), in comparison to only 14 patients (18.7%) whose TC levels were $\geq 190$ mg/dl (log-rank 26.306, $P<0.001$). In addition, patients with low LDL-C (<120 mg/dl) experienced 37 cardiac events (66.1%), whereas only 17 patients (18.9%) whose LDL-C was $\geq 120$ mg/dl had such events (log-rank 26.251, $P<0.001$). These data indicate that the upregulation of monocyte cytokine production and low cholesterol and LDL-C levels are significantly associated with poor outcome in patients with CHF.

Cox Proportional Hazards Analysis for Cardiac Events

The results of univariate and multivariate Cox hazard analysis are listed in Table 4. Multivariate analysis showed that production of monocyte TNF-$\alpha$ at baseline $>4.1$ pg·ml$^{-1}$·10$^{-6}$ PBMCs, BMI $<21.0$ kg/m$^2$, NYHA class III or IV, LVEF <26% and use of statins were each significantly and independently associated with cardiac events. The percent change, however, in production of TNF-$\alpha$ $>-5.2\%$ (HR, 4.56; 95% CI: 0.90–23.07, $P=0.067$) or of IL-6 $>-8.4\%$ (HR, 1.79; 95% CI: 0.62–5.21, $P=0.286$) from baseline at 6 months, was not an independent predictor of cardiac events (data not shown).

Table 4. Factors Predicting Cardiac Events

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR  95% CI</td>
<td>P value</td>
</tr>
<tr>
<td>TNF-$\alpha$ $\geq$ 4.1 pg·ml$^{-1}$·10$^{-6}$ PBMCs</td>
<td>32.38 10.09–103.80 &lt;0.001</td>
<td>28.37 3.53–227.8 0.002</td>
</tr>
<tr>
<td>IL-6 $\geq$ 160 pg·ml$^{-1}$·10$^{-6}$ PBMCs</td>
<td>3.75 2.04–6.90 &lt;0.001</td>
<td>5.47 0.87–34.44 0.071</td>
</tr>
<tr>
<td>Age $&gt;$70 years</td>
<td>2.33 1.35–4.02 0.002</td>
<td>1.50 0.34–6.65 0.590</td>
</tr>
<tr>
<td>BMI $&lt;$21 kg/m$^2$</td>
<td>6.64 3.69–11.94 &lt;0.001</td>
<td>5.20 1.32–20.50 0.018</td>
</tr>
<tr>
<td>Heart rate $&gt;$78 beats/min</td>
<td>1.74 1.01–2.99 0.045</td>
<td>3.60 0.76–16.98 0.105</td>
</tr>
<tr>
<td>NYHA class III or IV</td>
<td>10.35 5.33–20.11 &lt;0.001</td>
<td>17.03 3.79–76.55 &lt;0.001</td>
</tr>
<tr>
<td>LVEF $&lt;$26%</td>
<td>5.42 2.89–10.16 &lt;0.001</td>
<td>14.03 2.72–72.37 0.002</td>
</tr>
<tr>
<td>TC $&lt;$190mg/dl</td>
<td>4.04 2.24–7.27 &lt;0.001</td>
<td>1.85 0.29–11.95 0.519</td>
</tr>
<tr>
<td>LDL-C $&lt;$120 mg/dl</td>
<td>4.03 2.24–7.25 &lt;0.001</td>
<td>1.49 0.45–4.98 0.517</td>
</tr>
<tr>
<td>BNP $&gt;$600 pg/ml</td>
<td>10.71 5.05–22.75 &lt;0.001</td>
<td>4.67 0.77–28.26 0.094</td>
</tr>
<tr>
<td>Hb $&lt;$12.0g/dl</td>
<td>3.4 1.94–5.96 &lt;0.001</td>
<td>2.08 0.40–10.74 0.382</td>
</tr>
<tr>
<td>eGFR $&lt;$45 ml·min$^{-1}$·1.73m$^{-2}$</td>
<td>2.37 1.34–4.20 0.003</td>
<td>3.01 0.93–9.82 0.067</td>
</tr>
<tr>
<td>CRP $\geq$0.47 mg/dl</td>
<td>9.97 5.00–19.89 &lt;0.001</td>
<td>1.20 0.20–7.20 0.840</td>
</tr>
<tr>
<td>Statin use</td>
<td>0.38 0.20–0.72 0.003</td>
<td>0.14 0.02–0.97 0.046</td>
</tr>
<tr>
<td>$\beta$-blocker use</td>
<td>0.25 0.13–0.47 &lt;0.001</td>
<td>0.50 0.09–2.89 0.436</td>
</tr>
<tr>
<td>Spironolactone use</td>
<td>0.5 0.29–0.86 0.012</td>
<td>0.86 0.22–3.36 0.826</td>
</tr>
</tbody>
</table>

CI, confidence interval. Other abbreviations as in Tables 1,2.

Figure 3. Kaplan-Meier event-free curves and log-rank tests indicated that (A) statin therapy significantly improved the outcome in patients with chronic heart failure during the follow-up period (log-rank 10.108, $P=0.001$). In addition, (B) monocyte production of tumor necrosis factor (TNF)-$\alpha$ $\geq 4.1$ pg·ml$^{-1}$·10$^{-6}$ PBMCs (log-rank 47.924, $P<0.001$) was significantly related to increased cardiac event rates during the follow-up period.
Discussion

The present study has provided evidence that the production of monocyte pro-inflammatory cytokines is significantly upregulated in patients with CHF in comparison to normal subjects. Furthermore, the patients with cardiac events had significantly higher monocyte cytokine production than those without such events, and there were significant positive correlations between plasma CRP and BNP, and the production of monocyte TNF-α and IL-6. LVEF was significantly and negatively associated with the production of monocyte TNF-α and IL-6. In addition, the upregulation of monocyte TNF-α and IL-6 production predicted a poor outcome in patients with CHF. These data suggest that the upregulation of monocyte-derived pro-inflammatory cytokine production may play a significant role in the pathogenesis and exacerbation of CHF. Statin therapy attenuated the production of monocyte pro-inflammatory cytokines, and ameliorated the cardiac function, and might improve the long-term prognosis in CHF patients with dyslipidemia.

Mechanisms of Upregulation of Monocyte Pro-Inflammatory Cytokine Production

The mechanisms by which the pro-inflammatory cytokines produced by monocytes are increased in patients with CHF remain unclear. The production of monocyte cytokines, however, can be further enhanced by various stimuli, including angiotensin II, CRP, and pro-inflammatory cytokines themselves. We have previously shown that plasma CRP is elevated and is also associated with the production of monocyte TNF-α and IL-6 in CHF patients. Interestingly, the exposure to recombinant CRP upregulates the production of monocyte TNF-α and IL-6. In addition, we have previously shown that the production of monocyte TNF-α and IL-6 by exposure to recombinant CRP is significantly associated with plasma CRP. Therefore, increased plasma CRP may lead to increased production of monocyte TNF-α and IL-6 in patients with CHF.

Taken together, these findings suggest that increased plasma CRP, as well as the levels of pro-inflammatory cytokines themselves, and the combinations of these factors, may upregulate the production of monocyte cytokines in patients with CHF.

Mechanisms Underlying the Beneficial Effects of Statins in CHF

The mechanisms responsible for the beneficial effects of statins in the present study remain unclear, but anti-inflammatory and antioxidant effects, improvement in endothelial function, inhibition of neurohormonal activation, and the combination of these effects (pleiotropic effects) may play an important role in the treatment of CHF.

Statins lower plasma cholesterol by inhibiting 3-hydroxyl-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in the mevalonate pathway. The intermediate products in the mevalonate pathway, including isoprenoids, lead to the activation of various downstream intracellular signaling molecules of the guanosine triphosphate-binding proteins such as Rho, Ras, and Rac. These proteins are involved in the activation of pro-inflammatory cytokines (Rho), in cell proliferation and hypertrophy (Ras), and in the production of reactive oxygen species (Rac).

The reduction of the plasma levels of pro-inflammatory cytokines by statin therapy has been convincingly demonstrated in a clinical study. The percent change in the LVEF from baseline was significantly and inversely associated with change in the production of monocyte cytokines in the statin group, thus suggesting that the improvement of cardiac function may be, at least in part, due to the reduction of monocyte pro-inflammatory cytokines in CHF patients treated with statins. In addition, we have also shown that statins reduce the plasma level of CRP in CHF patients, thus indicating that statins have anti-inflammatory effects in CHF patients.

Increased oxidative stress plays a significant role in the progression and development of CHF. Statins reduce the production of reactive oxygen species in CHF patients. Node et al demonstrated that simvastatin significantly improved the forearm vasodilator response to reactive hyperemia, thus suggesting that it led to an improvement in the endothelial function.

Taken together, statins seem to have pleiotropic effects that may be beneficial in patients with CHF.

Effects of β-Blockers and Spironolactone on Outcome in CHF

Large-scale, placebo-controlled, randomized clinical trials have demonstrated that β-blockers reduce the mortality and morbidity in patients with CHF. In addition, spironolactone also reduced the morbidity and mortality in CHF patients. Therefore, both β-blockers and spironolactone are standard treatments in patients with CHF.

The present study showed that statin therapy improved the long-term prognosis in CHF patients with dyslipidemia after matching them for use of both β-blockers and spironolactone. But this result may be difficult to interpret because the patients had received β-blockers or spironolactone for at least 1 year before enrollment in the present study. In addition, the patients in the acute decompensated stage of CHF were excluded from the present study. Therefore, all of the eligible patients in the present study were stabilized on standard treatment, including β-blockers or spironolactone before enrollment.

Discrepancy in Randomized Clinical Trials

The 2 recent prospective randomized trials, GISSI-HF and CORONA suggested that statin therapy did not have any beneficial effect on the outcome, despite a significant reduction in LDL-C and high-sensitivity CRP.

The main differences between the CORONA and GISSI-HF trials and the present study are in the types of subjects and the LDL-C levels. The CORONA study enrolled only patients with ischemic heart failure, while 40% of the patients in the GISSI-HF trial and only 22% in the present study had ischemic heart failure. Moreover, the patients were older (73 years vs. 68 years vs. 65 years) and more symptomatic (NYHA class III or IV; 63% vs. 37% vs. 40%) in the CORONA trial than in the GISSI-HF trial and the present study. The LDL-C level at entry in the present study was also higher (151 mg/dl vs. 138 mg/dl vs. 122 mg/dl) than in the CORONA and GISSI-HF trials.

Horwick et al showed that patients with low TC (<190 mg/dl) and LDL-C (<120 mg/dl) had significantly poorer outcome compared to those with high TC (≥190 mg/dl) and LDL-C (≥120 mg/dl). The present study found that patients with TC <190 mg/dl and LDL-C <120 mg/dl had significantly poorer outcome compared to those with TC ≥190 mg/dl and LDL-C ≥120 mg/dl, exactly in accordance with the Horwick et al study. These data suggest that the best cut-offs for TC and LDL-C for predicting cardiac events may be 190 mg/dl and 120 mg/dl, respectively. Therefore, too much of a reduction in the TC and LDL-C levels by rosuvastatin may not be beneficial for patients with CHF.

LDL-C decreased from 151 mg/dl to 115 mg/dl after 6 months of treatment in the present study, whereas it decreased from
137 mg/dl to 76 mg/dl in the CORONA trial and decreased from 122 mg/dl to 83 mg/dl in the GISSI-HF trial, thus indicating that better outcomes might have been obtained if LDL-C had not been decreased as strongly as observed in the previous trials.

The reasons for the discrepancies between the present study and the 2 randomized clinical trials remain to be confirmed. In the present study, none of the patients had received statins prior to enrollment and only CHF patients with elevated LDL-C (dyslipidemia), a primary indication for statin use, received statins after enrollment, whereas only a small number of patients had elevated LDL-C in the GISSI-HF and CORONA trials. In addition, the present study was a small cohort study. These may be the reasons for the different results compared to the 2 randomized studies.

**Conclusion**

The production of pro-inflammatory cytokines by monocytes is significantly upregulated in patients with CHF in comparison to normal subjects. Low TC and LDL-C were associated with poor outcome in patients with CHF. Despite these findings, statin therapy might improve the overall long-term prognosis in CHF patients with dyslipidemia. Further studies are needed to elucidate the exact mechanisms involved in this pathophysiological pathway and to also develop therapeutic strategies to suppress worsening of CHF.

**Disclosures**

None.

**References**