Effect of Cardiac Rehabilitation and Statin Treatment on Anti-HSP Antibody Titers in Patients With Coronary Artery Disease After Percutaneous Coronary Intervention

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SUMMARY

Accumulating evidence suggests that higher antibody titers to heat shock proteins (HSPs) are associated with the development and severity of atherosclerosis. The aim of this study was to evaluate the impact of cardiac rehabilitation therapy (CRT) or statin treatment (STT) or a combination of both (COM) on anti-HSP antibodies in patients with coronary artery disease (CAD) after percutaneous coronary intervention (PCI). Clinical evaluation of subjects was performed both at the commencement and completion of the 14 weeks of treatment. CRT consisted of a supervised 6 weeks of exercise following hospital discharge and 8 weeks of home stay exercise. Patients assigned to statin therapy were treated with 80 mg per day of fluvastatin. Blood samples from 39 patients were analyzed for antibodies to HSP60 and HSP70 by ELISA. Biochemical parameters, including lipids, high-sensitivity C reactive protein (hsCRP), and interleukin-6 (IL-6), were also analyzed. We found that CRT and COM reduced antibody titers to HSP60 and HSP70 in CAD patients (by 3.79 and 10.00% of anti-HSP60, and by 5.74 and 3.45% of anti-HSP70, respectively) but statin treatment reduced only antibody titers to HSP70 (by 3.83%). There was a significant correlation between antibody titers to HSP60 versus HSP70. Considering the fact that antibody titers to HSPs are associated with the autoimmune process in CAD, CRT and COM have greater effects on reduction in autoimmune reaction after PCI than statin treatment. This reduction was accompanied by greater improvements in blood biochemical variables, such as lipids, hsCRP, and IL-6 after CRT and COM. (Int Heart J 2006; 47: 671-682)

Key words: Cardiac rehabilitation, Statin, Exercise, Anti-HSP antibody, Coronary artery disease, Percutaneous coronary intervention
ATHEROSCLEROSIS is considered to be a disease whose pathogenesis includes inflammatory and immunological mechanisms, including autoimmune reaction against heat shock proteins (HSPs). HSPs are produced by cells of the arterial wall to protect the cells against any damage in response to stress or injury, such as infection, mechanical stress, oxidative stress, and cytokine stimulation. HSPs are 20-24 kDa molecules with sequence homology among species from bacteria to humans, therefore, it has been proposed that the immune response against bacterial HSPs results in target endogenous HSPs, which cause endothelial injury, and accelerate atherosclerosis. Accumulating evidence has demonstrated that higher anti-HSP60, HSP65, and HSP70 antibodies were associated with the development and severity of atherosclerosis. Recent evidence suggests that lipid-lowering medication with a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin) improves coronary artery diseases (CAD). Ghayour-Mobarhan and colleagues suggested that the underlying mechanism for this is not fully understood, but is likely to involve a reduction in blood lipid concentration, improvement in endothelial function, an anti-inflammatory action, an anti-thrombotic action, or a combination of these. The present clinical study was designed to demonstrate whether statin treatment could affect antibody titers to HSP60, HSP65, and HSP70 in CAD patients. Cardiac rehabilitation (CR) programs are clinically useful for reducing cardiac risk factors and increasing the exercise tolerance of CAD patients following cardiac events. Research shows that CR and exercise training down regulate the overall cardiac risk factors, including high-sensitivity C-reactive protein (hsCRP), reduce body fat, improve lipid profiles, and enhance the exercise capacity of CAD patients. Supervised rehabilitative exercise for 3-6 months has been reported to increase peak oxygen uptake by 11-36%.

To the best of our knowledge, no study has investigated the effects of cardiac rehabilitation on antibody titers to HSPs in CAD patients. In this study, we evaluated the effect of intervention using cardiac rehabilitation therapy, statin therapy, or a combination of the 2, on antibody titers to HSPs in CAD patients.

METHODS

Subjects: Patients were recruited from the cardiac rehabilitation clinics at Inje University Paik Hospital (Korea). Sixty patients (men and women) with acute myocardial infarction (AMI) or unstable angina (UA) were enrolled in the study. All patients had successfully undergone a percutaneous coronary intervention (PCI), approximately 7-10 days before entry into the study and were referred by their physicians. The patients were assigned to one of 3 groups; combined cardiac rehabilitation therapy and statin therapy (COM), cardiac rehabilitation therapy
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only (CRT), or statin therapy only (STT). Thirty-nine patients completed the 14 weeks of the experimental program (COM, n = 15; CRT, n = 14; STT, n = 10). Exclusion criteria included left ventricular ejection fraction < 40%; a history of previous myocardial revascularization, ie, coronary artery bypass graft (CAGB), PCI; severe exercise-induced myocardial ischemia, exercise-induced malignant ventricular arrhythmia; skeletal vascular disease; smoking, and alteration of medication and/or cessation of diet during the experiment. In all patients, clinical evaluation was conducted at the commencement and at the completion of the 14 weeks treatment. All subjects of the cardiac rehabilitation program (COM and CRT) entered a program of 6 weeks of supervised cardiac rehabilitation following hospital discharge and 8 weeks of home stay exercise. All subjects were prescribed daily lipid lowering medication consisting of 100 mg aspirin and 75 mg clopidogrel throughout the experimental period. Patients assigned to STT were treated with 80 mg daily of fluvastatin for 14 weeks. Each patient received individual counseling, including information concerning heart disease, risk factor modification (life style modification), physical training, diet management, and stress management during the experiment. The subjects were treated in accordance with the Helsinki Declaration of 1975.

**Clinical examination:** Clinical examinations were conducted before commencement of the experiments to obtain baseline values and at the end of the 14 weeks. The participants were also instructed to refrain from exercise on the day before examination. Trained laboratory technicians performed the examinations, which consisted of the following: obtaining blood for chemistry analyses; measurement of blood pressure; anthropometry; and a symptom-limited exercise test on a treadmill. Blood pressure was measured with mercury manometers. Body mass index (BMI), used as an index of body fat, was calculated as weight in kilograms divided by height in meters squared. Cardiorespiratory capacity was measured with a symptom-limited treadmill exercise test according to a modified Bruce protocol before and 6 weeks after completing the experiment.

**Blood sampling:** Fasting blood samples were collected between 8:00 and 10:00 AM after a 12-hour fast by venipuncture of an antecubital vein. Blood was collected into a serum vacutainer tube and the tubes were centrifuged at site. Serum was stored for further chemical analyses.

**Lipid profiles and inflammatory markers:** Fasting lipid profiles consist of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Blood lipids were analyzed using a Cobas Mira chemistry analyzer (Roche). LDL-C was calculated using the Friedwald equation, except for TG > 400 mg/dL. High-sensitivity C reactive protein (hsCRP) and interleukin-6 (IL-6) were measured by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Cal-
biochem, R&D Systems, respectively). Plasma HSP antibody titers were measured by ELISA. Anti-human HSP60 and HSP70 commercial ELISA kits (EKS-650, EKS-750) were purchased from StressGen (Canada). Serial dilutions of anti-human HSP60 and HSP70 (IgG/IgA/IgM) isolated from pooled human sera were used as standard. Data obtained as optical density values were calculated to this standard.

**Cardiac rehabilitation therapy:** Cardiac rehabilitation therapy consisted of 2 stages: The first stage which lasted 6 weeks was supervised exercise training based on cardiopulmonary treadmill exercise testing at a hospital, and the second stage was a home-based and self-managed exercise training program lasting 8 weeks. The exercise training consisted of a warm-up period, 30-40 minutes on a treadmill or bicycle ergometer, and a cool-down period. The exercise intensity of the first stage was increased progressively from 50% to 85% of VO2max determined by symptom-limited treadmill exercise. After the initial 6 weeks, the patients performed the home-based and self-managed exercise training program according to their new cardiorespiratory capacity. Exercise was performed 3 days per week during the 14 week period.

**Statistical analysis:** Data are presented as the mean ± SEM. Categorical data were compared using the Cochran Q test and continuous variables were compared by one-way ANOVA. Comparisons between before and after variables were assessed by a paired t-test for normally distributed data, or by the Mann-Whitney U test for nonparametric data. The Spearman rank correlation test and linear regression were used to assess interrelationships among variables. A value of $P < 0.05$ was considered statistically significant.

**RESULTS**

**Patient characteristics:** The baseline characteristics of the subjects are shown in Table I. There were no significant differences in the distribution of other anthropometric variables among the 3 groups. Body mass index was also similar in the 3 groups. The ratio of acute myocardial infarction (AMI) to unstable angina (UA) in COM and STT was almost 1:1, whereas UA was more common in the CRT group.

**Serum lipids:** Treatment with fluvastatin, 80 mg daily, did not alter the serum lipid levels in CAD patients after revascularization. On the other hand, combined therapy with CRT and STT significantly improved HDL-C and LDL-C (14.8%, $P < 0.05$; 12.5%, $P < 0.05$, respectively), however, only CRT significantly increased HDL-C (12.4%, $P < 0.05$). There were no statistically significant changes in the levels of TC and TG in any group (Table II).
hsCRP and interleukin-6: High-sensitive CRP showed a downward trend in the 3 groups, however, only the combination of cardiac rehabilitation therapy and statin therapy reduced hsCRP significantly, from a baseline value of 0.356 ± 0.063 mg/mL to 0.114 ± 0.015 mg/mL (P < 0.01; Table II). The levels of IL-6 decreased significantly in all 3 groups, however, the extent of the level of statistical significance of the change in IL-6 was different among the 3 groups (COM, P < 0.001; CRT, P < 0.01; STT, P < 0.01; Table II).

Heat shock protein antibody titers: Overall, the 2 groups with cardiac rehabilitation therapy (COM and CRT) had a significant reduction in the antibody titers to HSP60 and HSP70 (10.00%, P < 0.05; 3.79%, P < 0.05, respectively in COM and 3.45%, P < 0.05; 5.74%, P < 0.05, respectively in CRT). Statin therapy reduced the antibody titer to HSP70 (3.83%, P < 0.05) but not that to HSP60 (Table II; Figures 1 and 2).

In addition to comparing the groups, we examined the changes in anti-

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**Table I.** Baseline Characteristics of the Study Patients

<table>
<thead>
<tr>
<th></th>
<th>COM (n = 15)</th>
<th>STT (n = 10)</th>
<th>CRT (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.3 ± 1.8</td>
<td>52.5 ± 4.0</td>
<td>60.6 ± 2.6</td>
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<tr>
<td>Men</td>
<td>12 (80.0%)</td>
<td>8 (80.0%)</td>
<td>8 (57.2%)</td>
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<tr>
<td>Women</td>
<td>3 (20.0%)</td>
<td>2 (20.0%)</td>
<td>6 (42.8%)</td>
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<tr>
<td>Height (cm)</td>
<td>163.8 ± 2.3</td>
<td>167.5 ± 2.7</td>
<td>163.0 ± 2.2</td>
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</tr>
<tr>
<td>Weight (kg)</td>
<td>68.4 ± 1.8</td>
<td>74.7 ± 3.4</td>
<td>68.8 ± 2.8</td>
<td>0.194</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>UA</td>
<td>8 (53.3%)</td>
<td>5 (50.0%)</td>
<td>11 (78.6%)</td>
<td>0.259</td>
</tr>
<tr>
<td>AMI</td>
<td>7 (46.7%)</td>
<td>5 (50.0%)</td>
<td>3 (21.4%)</td>
<td></td>
</tr>
<tr>
<td>RHR (bpm)</td>
<td>74.9 ± 3.3</td>
<td>73.0 ± 3.3</td>
<td>74.5 ± 2.8</td>
<td>0.912</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117.7 ± 4.4</td>
<td>123.2 ± 5.2</td>
<td>125.6 ± 5.2</td>
<td>0.477</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78.4 ± 2.3</td>
<td>85.3 ± 3.3</td>
<td>84.5 ± 1.6</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 0.9</td>
<td>26.6 ± 0.7</td>
<td>25.8 ± 0.7</td>
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<td>Medications</td>
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<tr>
<td>ACEI</td>
<td>9 (60.0%)</td>
<td>8 (80.0%)</td>
<td>5 (35.7%)</td>
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<tr>
<td>Beta-blockers</td>
<td>7 (46.7%)</td>
<td>5 (50.0%)</td>
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<td>CCB</td>
<td>4 (26.7%)</td>
<td>3 (30.0%)</td>
<td>3 (21.4%)</td>
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<td>Diuretics</td>
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<tr>
<td>Nitrates</td>
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<td>9 (90.0%)</td>
<td>9 (64.3%)</td>
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<td>-</td>
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<tr>
<td>Aspirin</td>
<td>15 (100%)</td>
<td>10 (100%)</td>
<td>14 (100%)</td>
<td>1</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>15 (100%)</td>
<td>10 (100%)</td>
<td>14 (100%)</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. Cochran Q test for categorical variables; one-way ANOVA test for continuous variables. COM indicates combination of cardiac rehabilitation and statin; STT, statin; CRT, cardiac rehabilitation; UA, unstable angina; AMI, acute myocardial infarction; RHR, resting heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; ACEI, angiotensin converting enzyme inhibitor; and CCB, calcium channel blocker.
HSP60 and anti-HSP70 antibody titers with respect to UA and AMI. The reductions in anti-HSP60 and anti-HSP70 antibody titers were more remarkable in UA than in AMI. Even though slight decreases in the levels of anti-HSP60 and anti-HSP70 in AMI patients were observed, there were no statistically significant differences (Table III).
The relationships among variables of the serum samples were analyzed. Total cholesterol levels were associated with TG ($r^2 = 0.07$, $P < 0.05$), HDL-C ($r^2 = 0.11$, $P < 0.01$), and LDL-C ($r^2 = 0.75$, $P < 0.001$). Triglyceride level was associated with HDL-C ($r^2 = 0.09$, $P < 0.01$). A significant positive correlation between anti-HSP60 antibody and anti-HSP70 antibody was found ($r^2 = 0.14$, $P < 0.001$; Figure 3). However, there was no relationship between inflammatory factors, anti-HSPs antibody titers, and lipids (data not shown).
DISCUSSION

Accumulating evidence has shown that high anti-HSP60 and anti-HSP70 antibody titers are associated with the development and severity of atherosclerosis.\(^{10-14}\) The main aim of the present study was to evaluate the impact of cardiac rehabilitation therapy, statin therapy, or a combination of both on anti-HSP antibody titers. The major finding is that cardiac rehabilitation in CAD patients caused a significant decrease in anti-HSP antibodies. On the contrary, statin therapy alone did not significantly decrease anti-HSP60 antibody titers. Our results conflicted with those of Ghayour-Mobarhan and colleagues who reported that statin treatment was associated with a reduction in antibody titers to HSP60, HSP65, and HSP70.\(^{15}\) We believe that this discrepancy may be explained by the underlying differences in the nature of the subjects. The subjects in this study were patients who had undergone revascularization (PCI) and were previously treated with lipid-lowering medication during the acute-phase (7-10 days). On the contrary, patients who had taken lipid-lowering agents previously were excluded in their study. Pretreatment with lipid-lowering drugs is supposed to stabilize the blood lipids, weaken the lipid-lowering effect of statins, and then decrease the anti-inflammatory and immunoregulatory potential of statins. Considering these different results from different patients, we hypothesized that the effect of statin therapy on anti-HSP antibody may be observed in patients who were not previously treated with lipid-lowering agents or in those with early CAD, but not in

![Figure 3. Relationship between anti-HSP60 and -HSP70 antibody titers in CAD patients.](image)
patients who were previously treated with these drugs.

This is the first reported investigation of the effect of CR on antibody titers to HSP60 and HSP70 from patients with CAD. Although the prognostic value of the HSP antibody titers for future cardiovascular events has not been clear until now, considering the evidence that there is an association of serum antibodies to HSPs with heart disease, we postulated that down-regulation of the antibody titers to HSPs might retard or reverse CAD. Based on the findings obtained in the present study, down-regulation of the antibody titers to HSPs by CR and ST, intervention consisting of cardiac rehabilitation and statin therapy in CAD patients was found to be of benefit.

It is well known that autoantigens, such as oxidized LDL, bacteria/virus (Chlamydia pneumoniae, helicobacter pylori, cytomegalovirus, herpesvirus), and HSPs mediate the autoimmune process in the development of atherosclerosis. HSP antibodies can cross-react with bacterial and viral pathogens such as Chlamydia pneumoniae, and also human HSPs due to their homology and induce cytotoxic damage in stressed endothelial cells. HSPs and anti-HSP antibodies have been shown to elicit the production of proinflammatory cytokines by macrophages and adhesion molecules by endothelial cells in various studies. There are some functional differences between HSP60 and HSP70 or between anti-HSP60 antibody and anti-HSP70 antibody, however, and their autoimmune reactions to HSPs in vascular tissue may contribute to atherosclerosis. We also found that there was an association between anti-HSP60 antibody titers and anti-HSP70 antibody titers, although the association was weak. This finding supports the recent results from Ghayour-Mobarhan, et al., but is in conflict with the results reported by Kocsis, et al who found that there was no association between the titers of HSP70 antibody and either the anti-HSP60 or anti-HSP65 titer. It is interesting to learn that there was no relation between antibody titers to HSPs and other inflammatory factors, ie, hsCRP and IL-6 (data not shown). This means that they are independent risk factors of CAD.

The reduction of cardiovascular risk by lifestyle changes, such as diet, weight loss, exercise, and smoking cessation has been well documented. A CR program refers to intervention designed to optimize a cardiac patient's physical, psychological, and social functioning in addition to stabilizing, slowing, or even reversing the progression of the underlying atherosclerotic processes, thereby reducing morbidity and mortality. This study shows that therapeutic aerobic exercise can produce significant improvement in cardiac risk factors, including lipids as well as inflammatory markers, even though there were no changes in TC and TG. Exercise is inversely linked to LDL-C concentration, blood pressure, body mass index, glucose tolerance, fibrinolytic activity, and all other risk factors for cardiovascular disease, in addition, exercise promotes the hemodynamic func-
tion of the heart.32) Our results are consistent with previous findings.

Also in this study, statin therapy, a proven therapy to reduce the risk factors of CAD, was compared with cardiac rehabilitation therapy. The novel finding from this is that the impact of statin therapy was not demonstrated in the rehabilitation phase of CAD patients, even though partial effects on the levels of IL-6 and antibody titer to HSP70 were observed. These results did not agree with those of numerous previous studies on statin treatment, most of which reported that statins lower low-density lipoprotein42) and cholesterol,33,34) and have anti-inflammatory properties, including reducing levels of hsCRP,35-37) antioxidant properties,38) and an immunoregulatory effect.39) Inami, et al investigated the effects of statins on oxidized low-density lipoprotein in patients with hypercholesterolemia. Their results showed that the reduction in oxidized low-density lipoprotein in the fluvastatin group was significantly higher than that in the pravastatin group (47.5% versus 25.2%, \( P = 0.033 \)).42) Lee, et al demonstrated that treatment with fluvastatin 80 mg daily produced significant reduction in coronary atherosclerotic events in post-PCI patients.40) Kinlay, et al reported that high-dose atorvastatin potentiated the decline in inflammation in patients with acute coronary syndromes.41) This discrepancy seems to result from the nature of the subjects used. As mentioned above, the subjects in the present study were patients who had undergone revascularization (PCI) and were previously treated with lipid-lowering medication during the acute-phase (7-10 days). Pretreatment using lipid lowering medication reduced and relieved lipids and inflammatory factors, as was evident from the baseline characteristics of the patients. Modification of the levels of IL-6 and antibody titer to HSP70 by statin treatment, without alteration of the levels of lipids, lead us to believe that these benefits of statin therapy are derived independently from the lipid-lowering and hsCRP-lowering properties.

The results showed the reductions in anti-HSP60 and anti-HSP70 antibody titers were more prominent in UA than AMI. This interesting finding may be explained by taking into consideration the difference in the degree of inflammation or severity of myocardial damage between the 2 diseases. The addition of other blood biochemical indicators of inflammation should assist in better understanding this observation.

This study has several limitations. First, it was not randomized, raising the possibility of selection bias, because of the patient's individual clinical characteristics. The nontherapeutic control population was not recruited. Although we instructed and counseled all patients with respect to diet during the experiment, we were not able to control dietary intake completely.

**Conclusions:** The results of this study demonstrate that cardiac rehabilitation therapy reduced antibody titers to HSP60 and HSP70 in CAD patients after PCI, while statin treatment reduced only antibody titers to HSP70. Considering the
fact that antibody titers to HSPs are associated with the autoimmune process in CAD, cardiac rehabilitation therapy has greater effect on a reduction in autoimmune reaction after PCI than statin therapy. This reduction was supported by improvements in blood biochemical variables, such as lipids, hsCRP, and IL-6 after cardiac rehabilitation therapy.

REFERENCES


