Angiotensin-Converting Enzyme Inhibitors and 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase in Cardiac Syndrome X
Role of Superoxide Dismutase Activity

Carmine Pizzi, MD; Olivia Manfrini, MD; Fiorella Fontana, MD; Raffaele Bugiardini, MD

Background—Morbidity of patients with Syndrome X (SX; chest pain and normal coronary angiograms) is high and is associated with continuing episodes of chest pain and hospitalization. Impairment of microvascular endothelial function caused by increased oxidative stress has been suggested to be a mechanism of the disease. Superoxide dismutase (SOD) is the major antioxidant enzyme system of the vascular wall. This study sought to establish whether combination treatment with ACE inhibitors and statins reduces oxidative stress and improves quality of life with patients with cardiac SX.

Methods and Results—Forty-five patients with SX were randomly assigned to receive either a combination of ramipril (10 mg/d) and atorvastatin (40 mg/d) or placebo for 6 months. We determined the activity of extracellular SOD and its relation to flow-dependent endothelium-mediated dilation (FMD) and quality of life (exercise capacity and score with Seattle Angina Questionnaire [SAQ]) before and after treatment. After 6 months, patients with SX who received atorvastatin and ramipril had significantly reduced \( P<0.001 \) SOD levels (188.1 \( \pm \) 29.6 U/mL). No significant changes were seen on placebo (262.9 \( \pm \) 48.8 U/mL). Reduction of SOD after therapy was negatively correlated with FMD \( (r=0.38; P=0.01) \) and positively with total cholesterol \( (r=0.56; P<0.001) \). At follow-up, patients taking atorvastatin and ramipril improved their quality of life both in terms of exercise duration (by 23.46%) and SAQ (by 64.1%).

Conclusions—Six months of therapy with atorvastatin and ramipril improves endothelial function and quality of life of patients with SX. Reduced SOD activity may reflect low superoxide anion production. Benefits of these drugs may be related to reduction of oxidative stress. (Circulation. 2004;109:53-58.)

Key Words: syndrome X ■ endothelium ■ free radicals

Coronary microcirculation abnormalities have been shown to play a key role in patients found to have chest pain as well angiographically normal epicardial vessels (cardiac Syndrome X, SX). These patients show ECG ischemia and often, transient myocardial perfusion abnormalities during exercise stress test. Coronary endothelial dysfunction\(^1\),\(^2\) has been advocated as a possible cause of SX. Endothelial dysfunction may increase release of reactive oxygen species, which may trigger production of cytokines, cell adhesion molecules, and growth factors.\(^3\) All of these factors in turn may induce inflammatory and proliferative changes in the vessel wall, which could lead to microvascular dysfunction.

Morbidity of patients with SX is high and is associated with continuing episodes of chest pain and hospital readmission. Increased production of reactive oxygen species and endothelial dysfunction may contribute to the development of ischemia and chest pain and may be important in maintaining the disorder over time.\(^4\) Intervention with antioxidant agents such as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), and ACE inhibitors (ACE-I) have been shown to counteract reactive oxygen species production and improve endothelial function in coronary artery disease.\(^5\),\(^6\) These drugs may work as well in patients with SX.

The aim of the present study was to assess the effects of combined treatment with statins and ACE-I on patients with SX. We hypothesized that benefits could be related to reduction of oxidative stress within the arterial wall that may be reflected by low superoxide dismutase (SOD) activity.

Methods

Study Population
We studied 45 patients (mean age, 58.6 \( \pm \) 9.1 years) with SX in a randomized, prospective, single-blind, placebo-controlled fashion. Ages ranged between 33 and 68 years. Criteria of inclusion were as follows: (1) typical chest pain at rest and/or on effort, (2) normal 12-lead ECG at rest, (3) ischemia-like ECG changes during exercise stress test (horizontal or downsloping ST-segment depression >0.1
mV), (4) myocardial reversible perfusion abnormalities during exercise stress as assessed by single-photon emission-computed tomography, (5) normal left and right ventricular function at rest as assessed by echocardiography, (6) absence of valvular heart disease and myocardial hypertrophy, and (7) normal coronary angiograms at visual analysis and absence of coronary artery spasm during intravenous ergonovine test. Patients were not included in the study if they had hyperlipidemia (cholesterol >220 mg/dL) or had been treated with statins or ACE-I for any reason. They were also excluded if they had malignancy, kidney or liver failure, ongoing drug or alcohol abuse, or systemic inflammatory diseases. Contraindications to ACE-I and statins were exclusion criteria as well. Thirty-two women were postmenopausal, which was the result of bilateral ovariectomy in 3 patients and aging in the others. None of the women were receiving estrogen therapy. Twenty healthy volunteers (between 38 and 68 years; mean age, 57.9 ± 8.3 years) served as control subjects. None of them had major risk factors for coronary artery disease or history of chest pain. Twenty patients with documented coronary artery disease (between 48 and 68 years; mean age, 58.6 ± 6.6 years) were used as additional control subjects. Control patients were asked to give blood samples for quantitative evaluation of the biochemical markers of oxidative stress. All patients gave their written informed consent to the study.

Study Protocol
After angiography, all patients with SX received standard treatment with diltiazem (180 mg daily), which was kept constant during the study period. Patients discontinued medications and placebo only for 48 hours before clinical and laboratory tests. Investigations were performed in the morning after overnight fasting. Blood for general hematologic analyses, including total, LDL, and HDL cholesterol, transaminases, and creatinine kinase, was collected according to routine procedures. Additional blood samples were drawn to measure SOD activity. Flow-mediated dilation (FMD) of the brachial artery was assessed by means of ultrasonography. All patients performed a baseline exercise stress test. They were then randomly assigned to treatment with atorvastatin and ramipril (22 patients) or placebo therapy (23 patients). Patients were instructed to take 3 capsules (drugs or placebo) per day, the first capsule (5 mg ramipril) in the morning after breakfast and the remaining 2 (one of 5 mg ramipril, the other of 40 mg atorvastatin) in the evening after dinner. Physicians who were blinded to the clinical characteristics of patients monitored random assignment. They were also responsible for drug supply and for its matching with placebo. Patients were followed up for 6 months. Cardiology research staff, through patient visit, chart review, and serial telephone contacts, collected clinical event data at 1, 3, and 6 months. Serum creatine kinase was measured at 1 and 3 months. Levels of 3 times more than the upper limit of normal range resulted in discharge from the study. Anginal symptoms were assessed at each visit by interview and Seattle Angina Questionnaires (SAQ). Therapy compliance was assessed by pill counts. Exercise stress test, FMD, and blood samples for measurement of SOD and lipid profile were repeated after 6 months’ follow-up.

Investigators who performed biochemical measurements, exercise stress tests, and FMD were blinded to treatment regimen.

Angina Questionnaires
Health status assessment was performed by the SAQ. The SAQ is a 19-item questionnaire for patients with coronary artery disease. The SAQ quantifies patients’ physical limitations caused by angina, frequency of and recent changes in symptoms, treatment satisfaction, and quality of life. Analyses score ranges from 0 to 100. Higher scores indicate better function or fewer symptoms.

Flow-Mediated Dilation of the Brachial Artery
Brachial artery 2-dimensional and pulsed Doppler flow velocity signals were obtained above the antecubital crease with a 7.0-MHz linear array transducer, using a vascular ultrasound system (Hewlett-Packard, SONOS 2000). We induced hyperemia (endothelium-dependent FMD) by inflating a blood pressure cuff on the proximal portion of the arm to occlude arterial flow (>300 mm Hg) for 5 minutes and then rapidly deflating the cuff. After pulsed Doppler recordings, hyperemic 2-dimensional images were obtained 60 seconds after cuff deflation. After a 15-minute rest period to allow restoration of baseline conditions, we assessed non–endothelium-dependent brachial artery dilation before and after administration of 0.4 mg sublingual nitroglycerin (NTG). Images were stored on a super-VHS videotape recorder for further analysis. Intraobserver variability for FMD measurement was 1.1 ± 0.7%.

Blood Sampling
After 30 minutes of rest, venous blood was collected in ethylenediamine-tetraacetic acid tubes (for SOD) and in serum separating gel tubes (for serum lipid) and immediately placed on ice. Plasma was separated within 15 minutes and frozen at −70°C. SOD was extracted from hemolyzed erythrocytes according to the method of McCord and Fridovich, and its activity was assayed as described by Misra and Fridovich. SOD activity was expressed as U/mL (normal range, 164 to 240). The coefficient of variation for determination of SOD activity was 8.1%.

Treadmill Test
The treadmill exercise test (CASE Marquette 12, Marquette Electronics) was performed according to standard Bruce protocol. The exercise test was terminated when any of the following end points were reached: physical exhaustion, progressive angina, ST-segment depression ≥0.3 mV, dyspnea, or severe arrhythmia.

Statistical Analysis
Values are presented as mean ± SD for continuous variables and absolute number (percentage) for categorical variables. Analysis of normality was performed with the use of the Kolmogorov-Smirnov test. Differences between baseline characteristics were performed by using the Student’s t test for continuous variables and χ² test for categorical variables. Two-way, repeated-measures ANOVA was used to compare biochemical, exercise stress test, and FMD variables between treatment groups (placebo versus atorvastatin and ramipril) and phases of the study (baseline versus 6 months). The probability value was the interaction among phases and groups. Correlation analysis was performed with the use of Pearson’s correlation coefficient. All statistical tests were performed with the use of SPSS–Win 10.1 (statistical package for social science, SPSS Inc). Values of P<0.05 were considered significant.

Results
All 45 patients completed the study. No side effects were observed during follow-up. Baseline demographic and clinical characteristics of patients treated with atorvastatin and ramipril or placebo are presented in Table 1. At baseline, systolic and diastolic blood pressure, total cholesterol, HDL and LDL cholesterol, and triglyceride levels (Table 1) were similar between the 2 groups. After 6 months, patients treated with atorvastatin and ramipril had reduced values of the above variables. The difference between treatment and placebo was significant for total cholesterol (P=0.005), LDL (P=0.008), HDL cholesterol (P=0.01), and triglyceride (P=0.003).

Chest Pain Episodes
The results of SAQ are shown in Table 2. At baseline, the number of chest pain episodes per month for patients treated with atorvastatin and ramipril was 14.0 ± 5.3. Their average duration was 16.8 ± 4.5 minutes. No significant difference was found, as compared with placebo group (number, 13.6 ± 5.0; duration, 14.6 ± 3.5 minutes). At 6-month follow-up
up, patients treated with atorvastatin and ramipril significantly reduced the number of chest pain episodes as compared with placebo (4.4±2.9 versus 9.2±2.7, *P*=0.004). Both groups were reported to have significantly improved quality of life. When considering the individual response to treatment, combination of atorvastatin and ramipril significantly improved chest pain episodes in 16 and had no effects in 6 patients. Placebo had more random effects. It was associated with a worsening of symptoms in 5, no effects in 11, and significant improvement in 7 patients.

Exercise Stress Test
The results of the exercise stress tests are shown in Table 3. Exercise duration at follow-up was 23.46% greater with atorvastatin and ramipril than at baseline. Time to peak exercise was 450±82.2 seconds at baseline and 555.6±84.6 seconds at follow-up (*P*<0.045). Atorvastatin and ramipril prevented chest pain and ST depression in 9 of 22 patients. Different results were obtained when placebo was given.

FMD of the Brachial Artery
At baseline, there were no significant differences in brachial artery diameter and NTG-induced dilation between groups. Brachial artery response to NTG remained unchanged after treatment with atorvastatin and ramipril or placebo. Baseline FMD was similar in the treatment (2.2±1.3%) and placebo groups (2.1±1.3%). After 6 months, patients who received drugs had significantly improved FMD (4.2±1.7%, *P*=0.001), as compared with placebo (2.3±1.2%). Improvement of FMD after therapy was positively correlated with anginal symptoms (*r*=0.49; *P*<0.001) and time to peak exercise (*r*=0.29; *P*<0.01).

Biochemical Markers
SOD concentrations were 268.4±53.7 U/mL (range, 156 to 386) in patients with SX, 170.0±19.8 U/mL (range, 148 to 232) in the healthy control subjects, and 119.4±21.9 U/mL (range, 92 to 182) in coronary artery disease (*P*<0.001 between SX and control subjects). At baseline, there was no difference between patients with SX treated with atorvastatin and ramipril or placebo (272.2±55.8 U/mL versus 264.7±52.6 U/mL). After 6 months of follow-up, SOD was significantly (*P*<0.001) reduced in patients treated with atorvastatin and ramipril (188.1±29.6 U/mL) but not with placebo (262.9±48.8 U/mL). After therapy, reduction of
Flow-mediated dilation of Brachial Artery at Baseline and After 6 Months of Treatment

<table>
<thead>
<tr>
<th>Atorvastatin+ Ramipril</th>
<th>Placebo</th>
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<tr>
<td><strong>Peak exercise</strong></td>
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<tr>
<td>Time, s</td>
<td>450.0±82.2</td>
<td>481.2±79.2</td>
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<tr>
<td>Baseline</td>
<td>450.0±82.2</td>
<td>481.2±79.2</td>
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<tr>
<td>6 mo</td>
<td>555.6±84.6</td>
<td>488.4±79.2</td>
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<tr>
<td>Rate-pressure product</td>
<td>24.56±21</td>
<td>25.2±2.5</td>
</tr>
<tr>
<td>Baseline</td>
<td>24.56±21</td>
<td>25.2±2.5</td>
</tr>
<tr>
<td>6 mo</td>
<td>24.47±1.8</td>
<td>25.3±2.5</td>
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<tr>
<td>ST depression, mV</td>
<td>0.21±0.6</td>
<td>0.22±0.8</td>
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<tr>
<td>Baseline</td>
<td>0.21±0.6</td>
<td>0.22±0.8</td>
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<tr>
<td>6 mo</td>
<td>0.12±0.3</td>
<td>0.21±0.8</td>
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Flow-mediated dilation of brachial artery

<table>
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<tr>
<th>Flow mediated dilation, %</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>2.2±1.3</td>
<td>2.1±1.3</td>
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<tr>
<td>6 mo</td>
<td>4.2±1.7</td>
<td>2.3±1.2</td>
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<tr>
<td>Brachial artery diameter, mm</td>
<td>4.1±0.7</td>
<td>4.0±0.8</td>
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<tr>
<td>Baseline</td>
<td>4.1±0.7</td>
<td>4.0±0.8</td>
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<tr>
<td>6 mo</td>
<td>4.2±0.8</td>
<td>4.1±0.8</td>
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<tr>
<td>Nitroglycerin, %</td>
<td>11.2±3.6</td>
<td>11.1±3.3</td>
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<tr>
<td>Baseline</td>
<td>11.2±3.6</td>
<td>11.1±3.3</td>
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<tr>
<td>6 mo</td>
<td>12.0±3.3</td>
<td>11.3±3.3</td>
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Probability values are reported for comparison by 2-way ANOVA, comparing differences attributable to treatment.

SOD was negatively correlated with changes in FMD ($r=0.38; P=0.01$), exercise capacity ($r=0.22; P=0.03$), and SAQ ($r=0.46; P<0.01$) and positively with changes in total cholesterol ($r=-0.56; P<0.001$).

Discussion

The present study shows that 6 months of therapy with atorvastatin and ramipril improves endothelial function (flow-dependent endothelium-mediated dilation) and quality of life (exercise capacity and daily life symptoms) of patients with SX. The inverse relation between SOD activity and FMD suggests that high SOD activity in SX may be a mechanism that counteracts impairment of endothelial function resulting from increased superoxide anion formation. Benefits of these drugs may be related to reduction of oxidative stress within the coronary circulation.

Myocardial Perfusion in Angina With Normal Angiograms

The finding of normal coronary arteriograms implies a highly favorable prognosis, although it does not establish immunity from a morbid cardiac event. The overwhelming majority (50% to 94%) of these patients continue to have chest discomfort similar to that for which they underwent coronary arteriography. More than half of the patients use antianginal drugs for many years without significant benefits. Among these patients, it is unknown the number who have cardiac pain presumed to be ischemic. The presence of ischemic-like ST-segment changes and abnormalities in myocardial perfusion detected by cardiovascular magnetic resonance during chest pain suggests that the pain could be due to ischemia. However, a number of studies demonstrated that pain can be evoked by electrical stimulation of the right ventricle and failed to show left ventricular dysfunction during angina and ST-segment depression, which generates doubts on the ischemic origin of chest pain, at least in a number of patients found to have normal angiograms. SX may well comprise a heterogeneous disorder, and differences between studies may be related to differences in patient selection. We tried to select a homogeneous group of patients on the basis of chest pain occurring during reversible myocardial perfusion defects. All of them had associated endothelial dysfunction.

Endothelial Dysfunction in Angina With Normal Angiograms

The disease could be due to underestimation of the extent of atherosclerosis by angiography or to “primary” microvascular dysfunction limiting coronary flow reserve during exercise. Atherosclerosis may be largely diffuse in the conduit vessel wall before a “significant lesion” could narrow the lumen, giving the image of normal angiograms in atherosclerotic subjects. Possible disorders confined to the microvasculature include abnormal responses to neuropeptide Y, elevated endothelin-1, and vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 levels. Many reports on these patients have identified impaired coronary flow responses to acetylcholine, which implies impairment of endothelium-dependent vasodilation. Endothelial dysfunction may reside at both epicardial and microvascular levels that may be consistent with both the above-mentioned hypotheses. Endothelial dysfunction could be, therefore, a common pathway of different causes leading to chest pain of patients with normal angiograms. It may be due to increased superoxide anion–mediated inactivation of nitric oxide, which causes oxidative stress. This may induce leukocyte activation and release of vasoconstrictor substances. All of these factors may adversely affect the coronary circulation and result in anginal symptoms or silent ischemia during noninvasive testing.

Efficacy of Atorvastatin and Ramipril

Rationale for use of statins and ACE-I in SX was their potent antioxidant and antiinflammatory properties. Recent observations indicate that the microcirculation is a crucial target for the pleiotropic actions of statins because of their important role in decreasing oxidative stress and vascular inflammation. Statins may restore endothelial function and blood flow. ACE-I may benefit coronary vascular bed as well. Renin–angiotensin system inhibition is associated with reduced free radical concentration. Also, it improves coronary flow reserve by bradykinin-mediated, nitric oxide–dependent mechanisms. The present study demonstrates that initiation of combined antioxidant therapy (statins and ACE-I) profoundly improves clinical outcome of patients with SX.
Treatment prevented chest pain and ST depression at follow-up exercise testing in 41% of patients. Patients who received atorvastatin and ramipril had significantly improved FMD. Restoration of endothelial function therefore may represent a mechanism whereby antioxidant therapy improved the quality of life.

**SOD Activity in Cardiac Syndrome X**

To estimate the contribution of oxidative stress to the development of the disease, we determined the SOD levels before and after treatment with atorvastatin and ramipril. SOD has recently been reported to be the major antioxidant enzyme system of the arterial vessel wall.\(^{26}\) At enrollment, SOD activity was increased in SX as compared with healthy control subjects and patients with coronary artery disease. After 6 months of therapy with atorvastatin and ramipril, its activity was in the normal range. Restoration of normal SOD activity paralleled improvement in FMD and was associated with a significant increase of exercise capacity. This does not necessarily establish a cause-effect relation but strongly suggests that increased SOD activity before treatment could be due to the need of counteracting excess superoxide anion formation. Reduced SOD activity after therapy may represent a compensatory mechanism that results from decreased oxidative stress and increased nitric oxide bioavailability.

The present study showed that vascular SOD activity is substantially reduced in patients with established coronary artery disease who are known to have impaired FMD. That was the opposite of what we observed in patients with SX. Different mechanisms appear, therefore, to be responsible for impaired FMD in coronary artery disease and patients with SX. Accordingly, high SOD levels in SX are not a unique phenomenon. Increased SOD activity is also documented in young subjects with familial hypercholesterolemia and subsequent increased oxidative stress caused by high lipid peroxidation.\(^{27}\) This report also confirmed that SOD activity was reduced in patients with coronary artery disease by measuring SOD in plasma and not in hemolyzed blood cells, as in the present study.

**Limitations**

A double-blinded, double-placebo design would have been theoretically preferable to the use of a single-blinded, double-placebo design. However, a matching ramipril-atorvastatin placebo was not available from the manufacturers. We believed that it was preferable to include a double placebo in a single-blinded fashion rather than no placebo at all. We determined the blinding status of 5 groups: (1) participants; (2) investigators who performed biochemical measurements; (3) physicians who performed exercise stress test and FMD; (4) data collectors; and (5) data analysts.\(^{28}\) An additional limitation of the study is the lack of patient groups treated with ACE-I and statin alone. Both drugs per se might have contributed to the antioxidant effects observed in our study. However, the aim of the study was not to demonstrate the superiority of one drug compared with the other, but that combination of antioxidative drugs that may exert clinical benefits in terms of quality of life in a subgroup of patients for whom many claims of therapies have been made over the years.

We were concerned with the difficulty of proving that oxidative stress was reduced and nitric oxide stores were replenished by therapy. Many assays are available for measurements of products of oxidative stress in humans, but no single assay may accurately reflect free radical generation. This may certainly represent a further limitation of the study. We determined SOD activity, which seems to be a sensitive method to measure superoxide anion production in humans.\(^{27}\) Superoxide anions may form hydroxyl radicals, thus contributing to cause oxidative stress.

**Conclusions**

Therapy of patients with SX is still problematic. Patients used diltiazem for 6 months, often without significant benefits. Combination of ramipril and atorvastatin may be a good addition to this therapy.

The results of this study support the hypothesis that endothelial dysfunction caused by enhanced oxidative stress could be a cause of angina in many patients who have normal angiograms.

**References**


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