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Increased Plasma Thioredoxin in Patients with Acute Myocardial Infarction

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Summary

Background and hypothesis: Thioredoxin is an important biomarker for oxidative stress. We investigated whether thioredoxin levels were elevated in patients with acute myocardial infarction (AMI) and were associated with the results of coronary reperfusion.

Methods: The present study determined plasma thioredoxin levels in 51 patients with AMI, 30 patients with stable exertional angina (SEA), and 30 patients with chest pain syndrome (CPS). Plasma sampling was performed on admission, at 12 h, 1 week, 2 weeks, and 4 weeks in patients with AMI, and after admission in patients with SEA and CPS.

Results: Plasma thioredoxin levels on admission were higher in patients with AMI than in those with SEA and CPS. Plasma thioredoxin levels in patients with AMI were decreased in 12 h without further change thereafter. However, thioredoxin levels in patients with AMI remained higher than in those with SEA. In multivariate analysis, higher levels of thioredoxin on admission were a risk factor for failure in emergent reperfusion therapy in patients with AMI independent of other factors.

Conclusion: Plasma thioredoxin levels are elevated in patients with AMI, and higher thioredoxin levels may predict subsequent failed coronary reperfusion therapy in patients with AMI.

Key words: acute myocardial infarction, reperfusion, oxidative stress, thioredoxin

Introduction

Oxidative stress is believed to play an important role in coronary artery disease.[1, 2] A previous study has shown that plasma/serum levels of thioredoxin are elevated under oxidative stress-associated disorders such as viral infections and ischemia reperfusion.[3] Recently, thioredoxin in human plasma has begun to be quantitatively measured as a marker of oxidative stress by a sandwich enzyme-linked immunosorbent assay (ELISA).[3, 4] Measuring the plasma/serum levels of thioredoxin is a good diagnostic marker for the host response to oxidative stress. However, there are no reports showing a change in plasma levels of thioredoxin in patients with acute myocardial infarction (AMI). In the present study, we examined the plasma thioredoxin levels in patients with AMI, stable exertional angina (SEA), and chest pain syndrome (CPS), and the relationship between oxidative stress and the results of intervention in patients with AMI.

Methods

Study Population

The AMI group consisted of 51 patients (36 men, 15 women; mean age 65 ± 12 years) who were admitted within 6 h after the onset of symptoms. The diagnosis of AMI was made on the basis of chest pain persisting for at least 30 min, ST-segment elevation of > 0.2 mV in at least two contiguous leads on a standard 12-lead electrocardiogram (ECG), and elevation of serum creatine kinase (CK) level more than twice the upper

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limit of normal. The average time from the onset of symptoms to admission was 2.6 ± 1.9 (mean ± standard deviation [SD]) h, ranging from 0.3 to 6 h. The present study included patients with SEA and CPS who were matched for age and gender with those with AMI during the study period. The SEA group consisted of 30 patients (20 men, 10 women; mean age 68 ± 9 years) who had typical exertional angina, ≥ 90% narrowing of the major coronary arteries, and no coronary spasm induced by acetylcholine.[5] The CPS group consisted of 30 patients (18 men, 12 women; mean age 63 ± 8 years) who had no significant coronary artery stenosis (< 25% of luminal diameter), and no coronary spasm was demonstrated by the intracoronary injection of acetylcholine.[5]

Coronary Angiography

Immediately after they were diagnosed with AMI, all patients underwent coronary reperfusion therapy with recombinant tissue-plasminogen activator (t-PA), rescue percutaneous transluminal coronary angiography (PTCA), or direct PTCA. All patients with AMI including those in the present study were diagnosed in our hospital and coronary reperfusion therapy was started within 30 min after diagnosis. Twenty-one patients were treated with thrombolytic therapy, 6 patients with thrombolytic therapy and rescue PTCA, and 24 patients with direct PTCA. Successful reperfusion therapy was defined as loss of chest pain and improvement of ST-segment elevation and patency in the infarct-related artery at repeat coronary angiography, or improvement in coronary flow to TIMI grade 3 after reperfusion and patency in the infarct-related artery at repeat coronary angiography. Failed reperfusion therapy was defined as coronary flow remaining at TIMI grade 0–2 after rescue PTCA, distal embolism or no reflow occurring in the infarct-related artery, or total occlusion occurring in the infarct-related artery as shown at repeat angiography. Furthermore, repeat coronary angiography was performed in all patients with AMI to assess the infarct-related artery at 4 weeks after admission. Written informed consent was obtained from each patient and the patient's family. The study protocol was in agreement with the guidelines of the ethics committees at our institutions.

Blood Samples

After admission, blood samples for plasma thioredoxin were obtained from an antecubital vein in patients with SEA and CPS. Blood samples in patients with AMI were obtained immediately after admission. At the time of sampling, the first 3 ml of blood were obtained for biochemical assessment, and the subsequent 4.5 ml of venous blood were collected in a sequential manner into an evacuated tube containing 0.5 ml of sodium citrate (0.13 mol/l, pH 7.5). All blood samples were immediately centrifuged at 3000 rpm for 10 min at 4˚C, and aliquots of samples were stored at −80˚C until analyzed. Venous blood samples were also taken 12 h after initiation of thrombolytic therapy and direct PTCA, and 1 week, 2 weeks, and 4 weeks thereafter in the same manner.

Sandwich ELISA for Thioredoxin

Two different kinds of antirecombinant thioredoxin murine monoclonal antibodies (ADF-11 and ADF-21) and a sandwich ELISA for human thioredoxin were provided by FujiRebio Co. Ltd., Tokyo, Japan, and used as previously described.[3,4] As a standard, serial dilution of 5–320 ng/ml of recombinant thioredoxin were used. Data were analyzed by a software SOFTmax Version 2.31 (Molecular Devices Corp., Sunnyvale, Calif., USA) by fitting four parameter logit-log transformations of standard recombinant thioredoxin.

Statistical Analysis

All data were given as mean ± SD. The error bars in the Figures show the SD of the mean. The comparisons of continuous data among the three groups were performed with 1-way analysis of variance (ANOVA) followed by Scheffé's test, and those between the two patient groups were performed with unpaired *t*-test. The frequency data among the three groups and between the two patient groups were compared by the chi-square test. Probability levels < 0.05 were considered to be statistically significant. To evaluate thioredoxin levels as an independent risk factor differing between the patients in whom reperfusion therapy was successful or failed, multiple logistic regression analysis was performed, using the following factors as categorical covariates: high levels of thioredoxin on admission (> 144.0 ng/ml, 75th percentile of the distribution of the thioredoxin level in the AMI group), low high-density lipoprotrein (HDL) cholesterol (< 35 mg/dl), time to admission (> 2 h, 50th percentile of the distribution), infarct-related artery (not the left anterior descending artery), multivessel disease (double- or triple-vessel disease), reperfusion therapy on admission (non PTCA), and max CK (> 3973 IU/l, 75th percentile of the distribution of the CK level in the AMI group).

Results

Patients Characteristics among the Three Groups

The clinical characteristics in the AMI, SEA, and CPS groups are shown in Table I. The AMI, SEA, and CPS groups were matched for age, gender, frequency of coronary risk factors, and lipids levels. The AMI and SEA groups were also matched for extent of coronary artery disease.

Assessment of Plasma Thioredoxin Levels among the Three Groups

Plasma thioredoxin levels (ng/ml) were higher in patients with AMI (103.4 ± 65.4) than in those with SEA (27.14 ± 13.4) and CPS (17.7 ± 8.6, $p < 0.001$, Fig. 1). There was no difference in the mean level of plasma thioredoxin between the SEA and CPS groups. The plasma thioredoxin levels in patients with AMI were decreased after admission (Fig. 2). However, the plasma thioredoxin levels were elevated after 4

TABLE I Patient characteristics in the three study groups

Characteristics	Acute myocardial infarction (n = 51)	Stable exertional angina (n = 30)	Chest pain syndrome (n = 30)
Age (years)			
Mean	65 ± 12	68 ± 9	63 ± 8
Range	33–82	43–83	47–80
Men / women	36/15	20/10	18/12
Hypertension	28	17	13
Smoking	33	13	14
Diabetes mellitus	18	16	8
Obesity	16	6	4
Total cholesterol (mg/dl)	210 ± 46	193 ± 34	189 ± 34
HDL cholesterol (mg/dl)	44 ± 15	49 ± 16	50 ± 13
Triglyceride (mg/dl)	155 ± 93	115 ± 52	118 ± 41
Number of coronary arteries narrowed >75%			
0-vessel	0	0	30
1-vessel	41	20	0
2-vessel	5	6	0
3-vessel	5	4	0

Abbreviation: HDL = high-density lipoprotein. Values are expressed as mean ± standard deviation or number.

weeks in patients with AMI compared with those with SEA and CPS ($p < 0.001$).

Patient Characteristics between the Success and Failure Groups in Patients with Acute Myocardial Infarction

The clinical characteristics in the success (n = 41) and failure (n = 10) groups of patients with AMI are shown in Table II. The failure group consisted of three patients with no reflow, two patients without reperfusion, three patients with distal embolism, and two patients with subtotal occlusion shown by repeat coronary angiography after 4 weeks. There were no differences in age, gender, extent of coronary artery disease, time to admission, infarct-related artery, reperfusion therapy, and max CK level. However, HDL cholesterol level was higher in the success group than in the failure group.

Comparison of Thioredoxin Levels between Patients in the Success Group and Those in the Failure Group

In multiple logistic regression analysis, high levels of thioredoxin (> 144.0 ng/ml) were the significant predictors of failure of reperfusion therapy independent of other risk factors in patients with AMI (Table III).

Discussion

Our study demonstrated for the first time that plasma thioredoxin levels were significantly increased in patients with AMI compared with those with SEA and CPS. The elevation of serum thioredoxin levels in patients with heart failure has been reported.[6] Although the precise mechanism of thioredoxin secretion is still not clarified, plasma levels of human thioredoxin are the response against oxidative stress.[7] There is a possibility that plasma thioredoxin levels were elevated by the inflammatory response against the oxidative stress in patients with AMI in the present study.

Recently, Takagi *et al.* have demonstrated that in human atherosclerotic specimens, thioredoxin and thioredoxin mRNA were enhanced in endothelial cells and macrophages in the atherosclerotic plaques.[8] We have reported that in directional coronary atherectomy specimens, the area of macrophage infiltration in the sections from patients with unstable angina and AMI was larger than that in patients with SEA, and tissue factor expression on macrophages was more frequently observed in these patients.[9] Therefore, one of the major origins

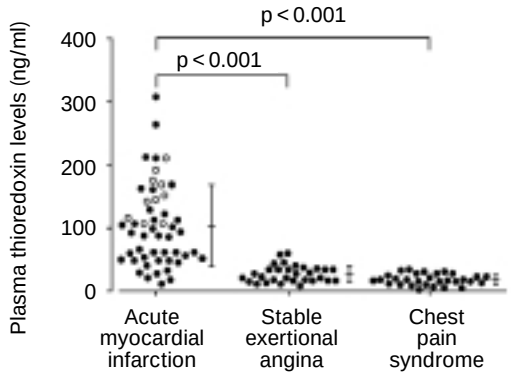

FIG. 1 Comparison of plasma thioredoxin levels among the acute myocardial infarction, stable exertional angina, and chest pain syndrome groups (mean ± standard deviation). Solid circles indicate patients with AMI in the failure group and open circles indicate patients with AMI in the success group. AMI = acute myocardial infarction.

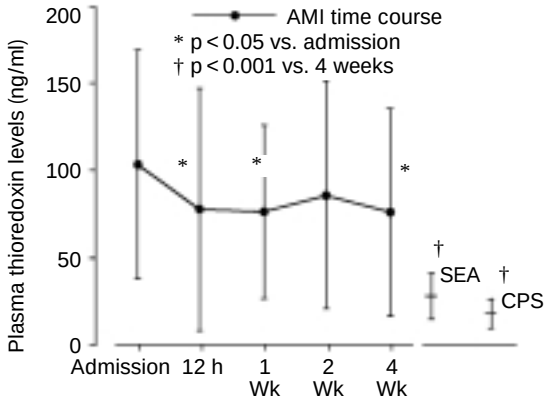

FIG. 2 Serial change in plasma thioredoxin level in patients with acute myocardial infarction (mean ± standard deviation). AMI = acute myocardial infarction, SEA = stable exertional angina, CPS = chest pain syndrome.

TABLE II Patient characteristics in the success and failure groups in patients with acute myocardial infarction

Characteristics	Success group (n = 41)	Failure group (n = 10)	p Value
Age (years)			
Mean	65 ± 13	65 ± 9	0.984
Range	53–82	33–82	
Men / women	27/14	9/1	0.133
Hypertension	23	5	0.729
Smoking	28	5	0.278
Diabetes mellitus	15	3	0.696
Obesity	12	2	0.556
Total cholesterol (mg/dl)	212 ± 47	198 ± 38	0.358
HDL cholesterol (mg/dl)	46 ± 15	35 ± 11	0.0424
Triglyceride (mg/dl)	155 ± 101	155 ± 58	0.988
Number of coronary arteries narrowed >75%			0.972
1-vessel	33	8	
2-vessel	3	2	
3-vessel	5	0	
Max CK (IU/l)	2604 ± 2214	2993 ± 1953	0.614
Infarct-related artery			0.106
LAD	19	4	
LCx	1	2	
RCA	21	4	
Therapy			0.205
PTCA	21	3	
Thrombolysis	17	4	
Rescue PTCA	3	3	
Time to admission	2.5 ± 1.9	3.2 ± 1.8	0.317
Thioredoxin levels on admission (ng/ml)	91.8 ± 66.1	150.8 ± 35.8	0.009

Abbreviations: HDL = high-density lipoproteins, CK = creatine kinase, LAD = left anterior descending, LCx = left circumflex, RCA = right coronary artery, PCTA = percutaneous transluminal angioplasty.

of elevated plasma thioredoxin is speculated to be unstable atheroscrelotic plaques in the infarct-related artery. It was also reported that the appearance of oxidative products in peripheral blood of patients with AMI is the result of their increased release from the infarcted heart during the inflammatory phase of myocardial ischemia.[10] Another major origin of thioredoxin may be ischemic or infarcted myocardium.

Several studies have shown that plasma levels of natural antioxidants are lower in cardiovascular diseases.[11,12] Low levels of antioxidants are thought to be the result of exhaustion of the antioxidants by increased lipid oxidation stress, such as free radical production induced by severe myocardial ischemia and reperfusion. Superoxide radical generation was detected in patients with ischemic heart disease.[13] The heightened plasma thioredoxin levels in the present study are consistent with such an increased oxidative stress in cardiovascular diseases.

In the present study, the thioredoxin level was higher in the failure than in the success group, and the HDL cholesterol level was higher in the success group than in the failure group. It was reported that antioxidants played a role in HDL cholesterol.[14] Oxidants influence the balance of the coagulation system toward platelet aggregation and thrombus formation.[15] Low HDL cholesterol is a coronary risk factor and may also be a prognostic factor of failed coronary reperfusion therapy. However, in the present study, only high levels of thioredoxin were the significant predictors of failure of reperfusion therapy independent of other risk factors in patients with AMI.

Glutathione supplementation reduced generation of reactive oxygen species in ischemia reperfusion in vivo.[16] Oxidative stress plays an important role in the mechanism(s) of endothelial dysfunction in cardiovascular diseases.[17–19] Antioxidants have been shown to restore endothelial function in patients with coronary artery disease.[20,21] It is known that statins and ACE inhibitors improve endothelial function;[22,23] therefore, they may influence the thioredoxin levels at admission and during hospital stay and follow-up. In the present study, there were no differences in medication between the success and failure groups. Further study may elucidate the relationship between thioredoxin and endothelial function in patients with coronary artery disease.

Data from many of our previously published studies on the subject of thioredoxin are cited in the present study.

Conclusion

We have demonstrated for the first time that plasma thioredoxin levels are elevated in patients with AMI compared with

TABLE III Multiple logistic regression analysis: Variables differing between the patients in the success group and those in the failure group

Charateristics	Odds ratio	95% CI	p Value
Thioredoxin on admission > 144.0 (ng/ml)	10.86	1.46–80.63	0.02
HDL cholesterol < 35 (mg/dl)	3.46	0.57–21.08	0.18
Time to admission (> 2 h)	3.41	0.48–24.45	0.22
Infarct-related artery (non LAD)	2.86	0.40–20.56	0.30
Multivessel disease	2.98	0.32–27.92	0.34
Reperfusion therapy (non PTCA)	2.07	0.33–13.01	0.44
Max CK > 3976 (IU/l)	1.48	0.23–9.38	0.68

Abbreviation: CI = confidence interval. Other abbreviations as in Table II.

those in patients with SEA and CPS, and that higher thioredoxin levels on admission are a risk factor for failure of coronary reperfusion therapy in patients with AMI. Thus, oxidative stress may play a possible role in the pathogenesis of AMI.

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