Oxidative DNA Damage and Antioxidant Defense After Reperfusion in Acute Myocardial Infarction

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Purpose: Myocardial damage mediated by oxidative stress during acute myocardial infarction (MI) has been suggested as an obstructive factor on recovery after an MI. 8-Hydroxydeoxyguanosine (8-OHdG) is a marker for oxidative DNA damage; superoxide dismutase (SOD) and glutathione peroxidase (G-Px) are major antioxidant enzymes. We determined changes in the plasma level of 8-OHdG and activities of SOD and G-Px in patients with MI and examined the relations between those changes and other cardiac markers.

Methods: Blood samples were taken at the beginning of the therapy, on the third day of hospitalization, and on the day patients were discharged home. Plasma level of 8-OHdG and SOD and G-Px activities were measured by enzyme-linked immunosorbent assay and spectrophotometric kits, respectively.

Results: 8-Hydroxydeoxyguanosine level at the beginning of the therapy was found to be decreased on the third day of therapy and on the day patients were discharged home. With respect to the treatment way, 8-OHdG level was found to be slightly decreased on the third day of therapy and then remained stable in the group treated with thrombolytic agents. However, 8-OHdG level was found to be sharply decreased on the third day of therapy in the group that underwent primary percutaneous transluminal coronary angioplasty. No significant relations were determined between those measured parameters and serum levels of cardiac markers.

Conclusion: Although not correlated with other cardiac markers, plasma level of 8-OHdG shows a decrease after reperfusion therapy in patients with MI, and primary percutaneous transluminal coronary angioplasty seems much more effective than thrombolytic therapy for providing a low level of 8-OHdG.

Key Words: acute myocardial infarction, 8-hydroxydeoxyguanosine, glutathione peroxidase, superoxide dismutase, primary percutaneous transluminal coronary angioplasty

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Acute myocardial infarction (AMI), which is a major cause of death, is essentially a ischemic/hypoxic injury to the heart muscle. Reperfusion salvages the jeopardized myocardium; however, reintroduction of O_2 to an ischemic tissue may be associated with reperfusion injury, which could adversely affect left ventricular function and diminish the beneficial effects of reperfusion.[1,2] Reactive oxygen species (ROS) formed during reperfusion are highly reactive molecules; they can cause permanent damage on proteins, lipids, and DNA, which leads to structural and functional alterations. Defense against ROS is provided by an antioxidant system that is capable of preventing excess radical production and neutralizing ROS. Superoxide dismutase (SOD) and glutathione peroxidase (G-Px) are major antioxidant enzymes. Although SOD and G-Px are cellular enzymes, they are available in the serum at a detectable level.[3]

8-Hydroxydeoxyguanosine (8-OHdG) is a product of oxidative DNA damage. When it escapes from repair mechanisms, it has a propensity to mispair during the replication, leading to an increased frequency of spontaneous mutations.[4] Oxidized nuclear DNA, in general, undergoes repair. Upon repair, 8-OHdG appears in peripheral blood and is excreted into urine without being further metabolized. 8-Hydroxydeoxyguanosine is considered to be an important biomarker for oxidative DNA damage induced by ROS.[5]

The purpose of this study was to assess changes in 8-OHdG level and SOD and G-Px activities in patients with AMI and to determine the relation between those changes and biochemical cardiac markers. It has been suggested that primary percutaneous transluminal coronary angioplasty (PTCA) increases oxidative stress by inducing inflammatory response.[6] Another aim of the present study was to compare PTCA and thrombolytic therapy with respect to oxidative DNA damage/antioxidant activity.

MATERIALS AND METHODS

The study subjects consisted of 28 consecutive patients (20 men, 8 women) with AMI who were admitted to the coronary care unit of the Cardiology Department, Cerrahpasa Medical Faculty Hospital. All of the patients had permanent chest pain on admission. Twenty-two of the patients were diagnosed with ST-segment elevation myocardial infarction (STEMI); 6 of the patients were diagnosed with non–ST-segment elevation myocardial infarction (NSTEMI) according to the European Society of Cardiology and American College of Cardiology criteria.[7] We excluded patients with renal insufficiency, acute and/or chronic inflammatory diseases, thyroid disorder, cancer, or Parkinson disease. The patients underwent treatment with platelet antiaggregants, statins, anticoagulants, β-blockers, nitrates, and angiotensin-converting enzyme inhibitors. Coronary angiography was performed in 23 patients of the studied group. Patients with STEMI underwent primary PTCA, if door-to-balloon time was less than 90 minutes; otherwise, thrombolytic therapy was preferred according to the recommendations of the American College of Cardiology/American Heart Association guidelines for patients with STEMI. Patients with NSTEMI did not undergo coronary intervention therapy during the hospitalization period. Demographic data, cardiovascular risk factors, history, electrocardiogram findings, complete ST resolution, Killip class, coronary angiographic data, reperfusion therapy, glycoprotein IIb/IIIa receptor blocker, and medications at admission were recorded. The control group consisted of 27 age-matched, normohypertensive, normolipidemic healthy volunteers (10 women, 17 men; mean age,

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58 years [SD, 6 years]; BMI = 27 kg/m^2 [SD, 3 kg/m^2]). A total of 3 subjects were smokers (≤5 cigarettes/day). None of the subjects were taking antioxidant vitamins or any drug. Institutional ethics committee approval was taken in accordance with the principles of the Declaration of Helsinki, and informed consent was obtained from each patient.

In patients with AMI, blood samples were taken in 3 hours after the start of the therapy, on the third day of the therapy and on the day patients were stable and discharged home (it has come up to sixth or seventh day of the therapy). Eight of the patients have undergone primary PTCA; 14 of the patients have been given thrombolytic therapy. Eight milliliters of venous blood samples was collected into heparinized tubes and centrifuged immediately. After centrifugation at 2000*g* for 10 minutes, plasma was removed and kept at −80°C until the time of analysis.

Plasma level of 8-OHdG was measured with a competitive enzyme-linked immunosorbent assay kit from Oxis (catalog no. 21026; Foster City, CA). To eliminate interfering substances, plasma samples were filtrated (molecular weight <10,000) just before analysis of 8-OHdG. Activities of SOD and G-Px were determined by spectrophotometric kits from Randox (catalog no. SD 125 and RS-504, respectively; Crumlin, UK). Total cholesterol and high- and low-density lipoprotein cholesterol levels were determined by spectrophotometric methods. Total creatine kinase (CK), creatine kinase–MB (CK-MB), lactate dehydrogenase (LDH), and troponin I levels were assessed using chemiluminescent immunometric assay, and C-reactive protein level was measured by nephelometric assay.

Statistical Analysis

Measured parameters are expressed as mean (SD). Statistical significance of changes in blood 8-OHdG levels and SOD and G-Px activities were evaluated by analysis of variance with Tukey test. When the patient group was divided into 2 subgroups with respect to the ST-segment elevation and with respect to the type of reperfusion therapy, that is, primary PTCA and thrombolytic therapy, comparisons between those groups were made using Mann-Whitney *U* test. Differences between groups were considered significant at $P < 0.05$ level. To analyze dependent variables, Spearman correlation coefficient was used.

RESULTS

Detailed patient demographics, clinical parameters, and angiography and laboratory test results are summarized in Table 1. In 3 hours after the start of the therapy, plasma 8-OHdG levels in the patients were significantly higher than those in the controls. 8-Hydroxydeoxyguanosine level was found to be decreased on the third day of the therapy and on the day patients were discharged home. However, it was still at a higher level than that in the controls on the day patients were discharged home (Table 2). Patients without ST elevation also exhibited high levels of 8-OHdG in 3 hours after the start of the therapy. With respect to ST elevation, there was not a significant difference between patients in any time for 8-OHdG and G-Px. In 3 hours after the start of the therapy, SOD activity in the patients with ST elevation was found to be lower than that in the patients without ST elevation (Table 3). In the patients with ST elevation, plasma levels of 8-OHdG decreased on the third day of the therapy, but in the patients without ST elevation, the levels were found to be decreased on the day patients were discharged home. With respect to the treatment way, 8-OHdG level was found to be slightly decreased on the third day of the therapy and then remained stable in the group treated with thrombolytic agents (Table 4). However, 8-OHdG level was found to be sharply decreased on the third day of therapy in the group that underwent primary PTCA. No significant relation was determined between those measured parameters and serum

TABLE 1. Patient Demographics, Clinical Parameters, and Angiography and Biochemical Test Results at Admission

AMI Group (n = 28)	
Demographic data	
Age, y	60 (SD, 10)
Hypertension	14
Type 2 diabetes mellitus	8
Obesity (BMI ≥30 kg/m^2)	8
Smoking	17
History of CAD	7
Previous stroke	5
Clinical data	
ST-segment elevation	22
Killip class II-IV	7
Anterior wall location	5
Ventricular arrhythmia	2
Atrial fibrillation	1
Reperfusion data	
Thrombolysis	14
Primary PTCA	8
Complete ST-segment resolution	19
Medication at admission	
Aspirin	28
β-Blocker	25
ACE inhibitor	25
Statin	25
Glycoprotein IIb/IIIa inhibitor	4
Clopidogrel	20
Culprit artery	
Left anterior descending artery	8
Circumflex artery	6
Right coronary artery	8
Intermedier artery	1
Coronary angiography	
1-Vessel	9
2-Vessel	3
3-Vessel	8
No obstructive lesion	3
Biochemistry	
Plasma total CPK, U/L	1125 (29–5734)
Plasma CK-MB, U/L	295 (4–4608)
Total cholesterol, mg/dL	183 (83–296)
LDL cholesterol, mg/dL	121 (74–237)
HDL cholesterol, mg/dL	34 (20–41)
Triglycerides, mg/dL	145 (43–397)
Troponin I, ng/mL	15 (0.01 to >100)
LDH, U/L	298 (34–1299)

Data are expressed in number of patients or in medians and interquartile ranges.

CAD indicates coronary artery disease; ACE, angiotensin-converting enzyme; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

TABLE 2. Measured Parameters in Patients With AMI and Controls

	Patient Group (n = 28)			
	3 h After Start of the Therapy	Third day of the Therapy	The Day Patients Discharged Home	Control Group (n = 27)
8-OHdG, ng/mL	12.65 (6.59)*	8.72 (5.66)*†	8.17 (4.23)*‡	3.08 (1.06)
SOD, U/mL	2.61 (0.52)	3.03 (1.08)	2.80 (0.49)	2.58 (0.42)
G-Px, U/L	0.94 (0.26)	0.91 (0.27)	0.98 (0.25)	1.06 (0.21)

Values are presented as mean (SD).

*$P < 0.001$ versus control group.

†$P < 0.05$ versus in 3 hours after the start of the therapy.

‡$P < 0.01$ versus in 3 hours after the start of the therapy.

levels of cardiac markers such as total CK, CK-MB, LDH, and troponin I.

DISCUSSION

The current study shows that the plasma levels of 8-OHdG at the beginning of the therapy in AMI patients are at a higher level than those in controls. These data provide evidence for the hypothesis that oxidative stress had already increased during the ischemic period in AMI. In rat models, increased level of 8-OHdG in the cardiomyocytes of myocardial infarction (MI) area was assigned.[8] Previously, Nagayoshi et al.[9] monitored urinary 8-OHdG level in patients with AMI. They have determined that urinary 8-OHdG/creatinine levels on admission were higher in the AMI group than those in the non–cardiac-event group (patients have angina pectoris but no coronary stenosis or spasm); urinary 8-OHdG peaked at 4 hours after reperfusion therapy and then decreased 24 hours after therapy. In the present study, serum 8-OHdG level was found to be decreased on the third day of the therapy and unchanged by the time the patients were discharged home. As a limitation of the present study, the time intervals for measurement of 8-OHdG were too large to determine the reperfusion-mediated elevation in 8-OHdG level, which has been shown by Nagayoshi et al.[9] at the fourth hour of reperfusion therapy. Increased ROS production leads to the formation of 8-OHdG adducts on DNA. Free 8-OHdG appears in serum as a result of DNA repair. Quantity of 8-OHdG adducts on DNA, which is measured by high-performance liquid chromatography technique,[10] is an indicator of oxidative DNA damage, but quantity of free 8-OHdG in plasma, which is measured by enzyme-linked immunosorbent assay,[11] is not only an indicator of oxidative DNA damage but also a marker of DNA repair activity. The anoxia secondary to ischemia results in a switch of myocardial metabolism to anaerobic glycolysis and increased formation of lactic acid. Not only does this switch in metabolism occur, but also the flow of substrates into the myocardium via the blood and removal of metabolic products from it are also greatly reduced. Accumulation of lactic acid leads to the development of severe acidosis. Because the enzyme activity is closely related with intracellular pH, accumulation of lactic acid results in a disturbance in enzymatic activity. Activities of DNA repair enzymes may be inhibited under this condition, and then DNA repair mechanisms may impair. According to our knowledge, increased serum level of 8-OHdG in the patients with AMI may reflect not only oxidative stress but also necrosis of cardiomyocytes. At that point, we can hypothesis that plasma level of 8-OHdG can be considered as a marker for the infarct size and severity of MI. Beyond doubt, to confirm this hypothesis, much more detailed studies with very short measurement intervals are necessary.

We did not determine a significant change in SOD and G-Px activities. Plasma SOD activity in patients in 3 hours after the start of the therapy was not significantly different from that in the control group. It was found to be increased on the third day of the therapy and then slightly decreased by the time the patient was discharged home, but none of these changes were statistically significant. Plasma G-Px activity in the patient group in 3 hours after the start of the therapy was found to be

TABLE 3. Measured Parameters in Patients With AMI With Respect to ST Elevation

	8-OHdG, ng/mL	SOD, U/mL	G-Px, U/L
Patients with ST elevation (n = 22)			
In 3 h after the start of the therapy	11.81 (6.14)	2.49 (0.49)*	0.94 (0.25)
Third day of the therapy	7.64 (4.26)†	3.07 (1.11)	0.95 (0.28)
The day patients were discharged home	8.40 (4.05)	2.78 (0.47)	0.99 (0.19)
Patients without ST elevation (n = 6)			
In 3 h after the start of the therapy	15.76 (7.83)	3.01 (0.50)	0.93 (0.34)
Third day of the therapy	12.68 (8.54)	2.85 (1.05)	0.77 (0.18)
The day patients were discharged home	7.34 (5.17)	2.88 (0.61)	0.96 (0.45)

Values are presented as mean (SD).

*$P < 0.05$ versus in 3 hours after the start of therapy in patients without ST elevation.

†$P < 0.05$ versus in 3 hours after the start of therapy in patients with ST elevation.

TABLE 4. Measured Parameters With Respect to the Way of Reperfusion

	8-OHdG, ng/mL	SOD, U/mL	G-Px, U/L
Patients treated by thrombolytic agent (n = 14)			
In 3 h after the start of the therapy	10.90 (6.26)	2.51 (0.49)	1.01 (0.27)
Third day of the therapy	8.94 (4.30)	3.27 (1.20)*	0.90 (0.26)
The day patients were discharged home	8.94 (4.28)	2.89 (0.50)	0.94 (0.18)
Patients treated by primary PTCA (n = 8)			
In 3 h after the start of the therapy	13.38 (5.98)	2.47 (0.52)	0.82 (0.18)
Third day of the therapy	5.36 (3.28)†‡	2.72 (0.89)	1.04 (0.30)
The day patients were discharged home	7.45 (3.70)	2.59 (0.35)	1.08 (0.18)

*$P < 0.05$ versus in 3 hours after the start of the therapy in patients treated by thrombolytic agent.

†$P < 0.01$ versus in 3 hours after the start of the therapy in patients treated by primary PTCA.

‡$P < 0.05$ versus third day of the therapy in patients treated by thrombolytic agent.

similar to G-Px activity in the control group and has not shown a serious change during the reperfusion therapy. Previous data examining G-Px and SOD activities in AMI are contradictory. Reduced erythrocyte G-Px and SOD activities in AMI have been reported by some investigators.[12,13] Simic et al.[12] have determined that patients had decreased SOD and G-Px activities in 3 hours after the start of the thrombolytic therapy as compared with healthy controls; in AMI patients without successful reperfusion, erythrocyte antioxidant enzyme activity remains low during the postinfarction period of 7 days; AMI patients with successful reperfusion have a significant increase in the activities of both SOD and G-Px within the first hour after thrombolysis, followed by a decrease until the third postinfarction day. Diaz-Araya et al.[14] have also determined lower SOD and G-Px activities in plasma from AMI patients as compared with controls, and as an agreement with our data, they have not determined a significant change in those activities after primary PTCA. On the contrary, Muzakova et al.[15] determined a stimulation in G-Px activity that reached its maximum 90 minutes after the onset of treatment and returned to the initial value after 18 hours. Bor et al.[16] have not found a significant difference between patients with AMI and controls for plasma G-Px activity, whereas erythrocyte G-Px activity was higher in patients than that in the controls. Tomado et al.[17] have studied plasma SOD activity in patients with AMI within 6 hours of symptoms onset. In the patients who had undergone PTCA, plasma SOD activity increased at the first hour, returning to the basal level by 8 hours after PTCA. Plasma SOD activity did not significantly change in patients with unsuccessful PTCA or those with the no-reflow phenomenon. With respect to all those data, we could not determine a change in SOD and G-Px activities because of the large measurement intervals. However, in agreement with our data, Dusinovic et al.[18] have not recorded a significant change in SOD and G-Px activities in blood samples taken before and 1, 3, 6, and 24 hours after the institution of thrombolytic therapy in patients with AMI.

After reperfusion by primary PTCA, an immediate induction in oxidative stress and inflammatory response were determined in AMI.[6,19] According to Berg et al.,[19] 8-Iso-PGF2α (an indicator of oxidative stress–mediated lipid peroxidation) increased after restoration of blood flow, returned to initial values after 3 hours, and decreased below the initial value the following day, and there was no significant correlation between 8-iso-PGF2α and troponin T values. Toth-Zsamboki et al.[20] reported that primary PTCA led to an explicit rapid increase in 8-OHdG level, but 8-OHdG levels were normalized within 96 hours. According to our data, the way of providing reperfusion seems to have a reliable role on the level of 8-OHdG. 8-Hydroxydeoxyguanosine level was found to be moderately decreased on the third day of reperfusion and then remained stable in the group treated with thrombolytic agent. However, 8-OHdG level has shown a sharp decrease on the third day of reperfusion in the group that underwent PTCA. Although 8-OHdG level slightly increased on the day patients were discharged home, it was still lower than that in the group treated with thrombolytic agent. If high plasma level of 8-OHdG is derived from necrotized infarct area, primary PTCA seems much more effective than thrombolytic therapy for providing reperfusion.

In conclusion, our data reveal that plasma level of 8-OHdG shows a significant decrease after reperfusion therapy in patients with AMI. 8-Hydroxydeoxyguanosine level in plasma may be a new independent marker for progression of MI. To confirm this hypothesis, much more detailed studies with shorter measurement intervals in larger patient groups are needed.

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