Decreased Glutathione Levels in Acute Myocardial Infarction

Ayhan USAL, MD, Esmeray ACARTURK, MD,
Güneş T. YÜREGİR, PhD, İsa ÜNLÜKURT, MS,
Celal DEMİRÇİ, MS, Halil İ. KURT, MD,
and Ahmet BİRAND, M.D.

SUMMARY

Although experimental studies have demonstrated that reduced glutathione (GSH) is involved in cellular protection from deleterious effects of oxygen free radicals (OFRs) in ischemia and reperfusion, there are controversial data on the correlation between the levels of erythrocyte GSH and the ischemic process. To clarify, we determined the erythrocyte GSH levels in 21 patients with acute myocardial infarction (AMI), aged 39–70, who were not given thrombolytic therapy and 21 age- and sex- matched healthy controls. Samples of blood were taken on days 1, 3, 5 and 7 from AMI patients and on the same days from the controls.

The GSH levels of patients with AMI were significantly depressed by 11.5% as compared to the controls on the second day after infarction (7.44 ± 1.71 vs 8.41 ± 1.54 U/gHb $p < 0.05$). Although the total mean of GSH levels for all days was lower (3.8%) in patients than in the controls, this finding did not reach statistical significance (7.41 ± 1.71 vs 7.71 ± 1.27 U/gHb, ns). There was no correlation between the erythrocyte GSH levels and cardiac enzyme concentrations, infarct localization, hemodynamic status according to Killip classification and the frequency of ventricular arrhythmias.

This preliminary work suggests that depressed GSH levels may be associated with an enhanced protective mechanism to oxidative stress in AMI. Measurements of erythrocyte GSH can be helpful in the estimation of oxidative stress in the course of AMI. However, further research must be done to determine the primary scavenger in AMI by analyzing all the enzymes and substrates involved in the endogenous system that controls the effects of OFRs. (Jpn Heart J 37: 177–182, 1996)

Key words: Myocardial infarction Glutathione

GLUTATHIONE (GSH) is a low-molecular weight endogenous sulfhydryl-containing tripeptide which is involved in protection of the cell against oxygen free radical (OFR) injury.¹⁾ *In vitro* and *in vivo* studies on OFRs suggest that

From the Departments of Cardiology and Biochemistry, Medical Faculty, Çukurova University, Adana, Turkey.

Address for correspondence: Ayhan Usal, MD, Yenibaraj Mh. 1778 Sk. Coskuner Apt., A Blok No: 15, 01150 Adana, Turkey.

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OFRs are toxic to the myocardium and can cause tissue damage that results in extensive necrosis, myocytolysis and cellular edema.^{2,3)} Among the enzymatic systems capable of protecting the cell against OFR injury, GSH, GSH peroxidase (GSHP), GSH reductase (GSSGR) and GSH transferase play a crucial role.^{4,5)} When hydrogen peroxide and lipid peroxides accumulate, GSH is oxidized to glutathione disulfide (GSSG) through the action of GSHP. If necessary, GSH is regenerated from GSSG by GSSGR and its cofactor, NADPH.⁶⁾ Under conditions of increased production of OFRs, tissue GSH content may be inadequate to prevent the oxidation of membrane lipids.⁶⁾ Blaustein et al⁴⁾ demonstrated that GSH is important in protecting the myocardium against OFR injury and that a reduction in cellular GSH content would impair recovery after short periods of ischemia. Although other experimental studies support this observation, there is a paucity of clinical data based on this concept.⁷⁻⁹⁾ Since the erythrocyte GSH reflects cardiac levels, we determined the erythrocyte GSH level changes in patients with acute myocardial infarction (AMI) and investigated the association between GSH status and the clinical course of AMI.¹⁰⁾

PATIENTS AND METHODS

Twenty-one patients with AMI were prospectively studied. All patients had their first acute myocardial infarction. Patients were eligible for the study when the following diagnostic criteria were present: Typical chest pain, specific abnormalities for AMI on the electrocardiogram, elevated serum total creatine phosphokinase (CPK) and CPK-MB and aspartate amino transferase enzyme levels. Patients older than 75 years and patients with clinical evidence of chronic heart disease (rheumatic or congenital heart disease or cardiomyopathy), diabetes mellitus, chronic renal or hepatic disease, severe anemia, infection and thyrotoxicosis were excluded from the study. Patients were treated with aspirin, nitrates, β blockers and/or calcium antagonists. Twenty-one age- and sex-matched healthy volunteers were also studied as controls. Smoking habit, systolic and diastolic blood pressures and family history of coronary heart disease (CHD) were recorded after clinical confirmation of AMI. The clinical progression of the patients, the site of infarction, development of arrhythmias and the hemodynamic status of the patients were followed. Ventricular arrhythmias were classified according to Lown criteria. Patients' hemodynamic status was evaluated according to the Killip classification.

Laboratory analysis: Venous blood samples were obtained from the patients on days 1, 3, 5 and 7 after AMI and on the same days from the controls. Blood samples were immediately taken into plastic tubes containing EDTA and placed in ice. Samples were studied immediately in order to avoid GSH oxidation.

Reduced GSH concentration in erythrocytes was measured using the method of Beutler.¹¹⁾ Activity is reported as units per gram of hemoglobin (U/gHb). Serum triglyceride, total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, glucose, blood urea nitrogen (BUN) levels were also determined in all patients and controls.

Statistical analysis: We compared the daily changes in GSH levels in patients with AMI and the controls by ANOVA for repeated measurements. The data from patients and controls were also compared by Student's *t* test. Values are expressed as means $\pm$ standard deviation; $p < 0.05$ was considered to be statistically significant.

RESULTS

The main clinical characteristics of the patients and the controls are presented in Table I. All patients had Q wave transmural myocardial infarction. None of the patients had coronary angioplasty or thrombolytic therapy.

Erythrocyte GSH levels were lower in patients with AMI than in the controls (Table II). On the second day of AMI, GSH levels of the patients were significantly depressed by 11.5% as compared to controls (7.44 ± 1.8 vs 8.41 ± 1.54 U/gHb, $p < 0.05$). Although the total mean level of GSH for all days was lower (3.8%) in patients as compared to the controls, this effect did not reach statistical significance (7.41 ± 1.71 vs 7.71 ± 1.27 U/gHb, $p > 0.05$). ANOVA for repeated measurements showed no difference between admission and subsequent levels of GSH in patients with AMI (ns) (Table II). There were no correlations between erythrocyte GSH levels and cardiac enzyme concentrations, infarct lo-

Table I. Clinical Characteristics of the Patients

Number	21
Age	39-70 (52 ± 9)
Sex	19M, 2F
Cigarette smokers	15
History of hypertension	11
Family history of CHD	6
Site of AMI	
Anterior	13
Inferioposterior	8
Ventricular arrhythmias	
Lown Class I	14
Class II	6
Class IV	1
Hemodynamic status	
Killip Class I	15
Class II	5
Class III	1

Table II. Erythrocyte GSH Levels in Patients and Healthy Controls

Days	Patients (n = 21)	Controls (n = 21)	
1	7.58 ± 1.71	8.18 ± 1.08	ns
2	7.44 ± 1.80	8.41 ± 1.54	< 0.05
3	7.46 ± 2.01	7.48 ± 1.28	ns
5	7.27 ± 1.56	7.11 ± 1.02	ns
7	7.33 ± 1.49	7.37 ± 1.40	ns

calization, hemodynamic status and the frequency of arrhythmias.

DISCUSSION

Indirect evidence of OFR generation in patients with AMI has been obtained by measuring a variety of by-products of lipid peroxidation, such as pentane, conjugated dienes, and malondialdehyde (MDA). The plasma concentrations of these substances were elevated in patients with AMI.^{9,12} OFRs are known to be able to bring about multiple changes in cellular structure and function.² Evidence from studies in animals suggest that the extent of myocardial necrosis and deterioration of myocardial function can be affected favorably by treatment with OFR scavengers.³ Among the OFR scavengers GSH is involved in prevention of OFR formation and accompanying tissue injury by acting as a substrate for GSHP during the reduction of hydrogen peroxide.^{1,13} Myocardial ischemia and reperfusion lead to reduction in the tissue content of GSH and protection against oxidant injury correlates with tissue levels of this substance.¹³⁻¹⁵ Previously, Ferrari et al¹³ observed a positive correlation between the duration of ischemia in patients undergoing cardiopulmonary by-pass and the efflux of oxidized GSH. Recently, Dubois-Rande et al⁹ showed that plasma oxidative stress was evident in patients with unstable angina pectoris (UAP) and AMI. They reported that erythrocyte GSH levels were significantly depressed in AMI and UAP as compared to controls. Similarly to these investigators we found a decrease in erythrocyte GSH levels in patients with AMI, particularly on the second day of infarction. Our results are consistent with these studies demonstrating the role of GSH in scavenging OFRs, especially in the early stage of AMI.^{9,14,15} Since OFRs are important mediators of reversible myocardial injury and GSH plays a role in scavenging activity of OFRs, the decreased GSH levels in our patients with myocardial infarction seem to be secondary to an enhanced antioxidative mechanism of protection against OFRs.^{16,17} OFRs are generated particularly in the early stage of AMI and endogenous GSH is utilized for reduction of hydrogen peroxide radicals, resulting in a decrease in GSH levels during that period.⁴⁻⁶ Physiologically, when tissue levels of GSH reach their minimum, GSH

is regenerated from endogenous glutathione disulfide through the action of glutathione reductase and its cofactor NADPH.⁴⁻⁶⁾ As a result of this reaction, endogenous GSH concentrations tend to increase to their normal levels. Probably due to that defense mechanism of the organism, the erythrocyte GSH levels of our patients with AMI did not significantly differ from those of the controls except on the second day of infarction.

Despite the demonstrable toxicity of OFRs to myocytes and the apparently protective effects of free radical scavengers in studies of animal models, salvage of the jeopardized myocardium by interventions attenuating OFR production has been inconsistent.¹⁸⁾ Some OFR scavengers such as superoxide dismutase (SOD), catalase and GSHP as well as antioxidants such as GSH, alpha-tocopherol and the GSH precursor N-acetylcysteine have been tried as therapeutic approaches for OFR-induced myocardial injury.^{2,19)} Treatment with exogenous GSH had antiarrhythmic effects and effectively reverse the deterioration in cardiac function.^{3,16)} Di-Pasquale et al¹⁶⁾ reported that when patients with AMI received GSH in addition to thrombolysis, they developed less frequent ventricular arrhythmias and better ejection fraction compared to patients treated with thrombolysis alone. Furthermore, myocardial GSH depletion impairs recovery of left ventricular function.^{4,7)} However, the beneficial effects of GSH are particularly evident in myocardial ischemia with reperfusion.⁸⁾ In canine permanent myocardial ischemia without reperfusion, Such et al⁹⁾ showed that exogenous GSH had only a minor beneficial effect on myocardial salvage after permanent coronary occlusion. In this study, we did not observe any correlation between GSH levels and the site of infarction, hemodynamic status or development of ventricular arrhythmias in patients with AMI. Since patients with coronary angioplasty or thrombolytic therapy were not included in the study, this observation might be due to the limited role of endogenous GSH in protecting the myocardium against ischemia without reperfusion.^{7,8)} However, the results of our study have included only the measurement of endogenous GSH, and are not sufficient to give any information about the effects of exogenous GSH either on ventricular function or development of arrhythmias.

Our data suggest that GSH levels are decreased in patients with AMI, particularly in the early stage of infarction. Depressed GSH levels may be associated with an enhanced protective mechanism to oxidative stress in AMI. Therefore, measurements of erythrocyte GSH can be helpful in the estimation of the extent of AMI. Further research including simultaneous measurements of all enzymes and substrates involved in the endogenous system that controls the effects of OFRs and comparative studies in patients with and without revascularization with coronary angioplasty or thrombolytic therapy are necessary in order to arrive at final conclusions about the role of GSH in AMI.

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