Oxidative and Antioxidative Status after Successful Percutaneous Transluminal Coronary Angioplasty in Acute Myocardial Infarction

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Abstract

Background: Oxidants lead to cell death through apoptosis and necrosis. The effects of reactive oxygen radicals are balanced by the antioxidant action. The aim of this study was to assess and compare the oxidative and antioxidative status, both in healthy control subjects and in patients with acute myocardial infarction after successful Percutaneous Transluminal Coronary Angioplasty (PTCA).

Materials and methods: 25 patients who were re-vascularised in the first six hours of Acute ST elevated myocardial infarction and 25 healthy controls were included in the study. Erythrocyte Total Superoxide Scavenger Activity (TSSA), Non-enzymatic Superoxide Scavenger Activity (NSSA), superoxide dismutase (SOD), Nitric Oxide (NO), glutathione peroxidase (GPX) activities and glutathione (GSH), malondialdehyde (MDA) and 8 hydroxydeoxyguanosine (8-OHdG) levels were measured before PTCA (zero-time) after the 6th hour and 24th hour from PTCA in patients and once in the controls.

Results: There was a decrease at MDA concentrations (3.99 ± 1.14 , 4.05 ± 1.35 , 3.62 ± 1.11 nmol/g Hb, $p < 0.05$), however, 8-OHdG concentrations significantly increased in patient at zero-time and 6th hours after PTCA (2.52 ± 1.27 , 2.56 ± 1.36 10^6 dG, $p < 0.001$) and a lower significant value at the 24th hour (2.50 ± 1.63 10^6 dG, $p = 0.001$). TSSA, NSSA, SOD, NO, GPX, GSH significantly increased in patients in zero-time, 6th hour and 24th hour of acute myocardial infarction. After PTCA an increase of concentrations continued.

Conclusion: Prior studies reported an imbalance in the favour of oxidative stress in acute myocardial infarction after reperfusion. This study showed that PTCA and routine medical therapy downgrades the imbalance of oxidative and Anti oxidative status in favour of the Anti oxidative effect.

Keywords: Acute myocardial infarction; Oxidative stress; Antioxidant defence; Percutaneous transluminal coronary angioplasty

release of materials stimulating platelet activation and thrombin formation. As a result, a thrombus limiting blood flow is formed. This situation leads to an imbalance between oxygen demand and supply, and if it becomes permanent, it leads to acute myocardial infarction [1]. Previous studies have shown that the imbalance between formation of reactive oxygen species and antioxidant defence together with conventional risk factors play an important role in the pathogenesis of atherosclerosis. The production of reactive oxygen species start during ischemia and go on even after reperfusion. The excessive production of reactive oxygen species plays a role in the pathogenesis of atherosclerosis leading to oxidation of low density lipoproteins, endothelial dysfunction, growth of vascular smooth muscle and monocyte migration [2]. Percutaneous Transluminal Coronary Angioplasty (PTCA) is the leading method among mechanical revascularisation procedures. Reperfusion after ischemia can increase the production of oxidants, which can overcome antioxidant capacity. Some previous studies showed that an increase in the production of oxidants with a decrease in the production of antioxidants could cause an imbalance in favour of oxidants. Increase of oxidants, leading lipid peroxidation in cell membrane, damage of membrane proteins and, DNA fragmentation with myocardial cell injury, causes loss of contractile function. In some in vitro studies, a burst-like increase in free oxygen radicals after ischemia and reperfusion in an isolated heart was reported. Bolli R, et al. [3] found that free oxygen radicals increased in significant amounts in coronary venous blood after 15 minutes of coronary occlusion and reperfusion [3]. These studies reveal that the production of free oxygen radicals continues and increases significantly during reperfusion after ischemia. Studies on oxidative stress during PTCA have produced controversial results [4]. In our study we aimed to show oxidative and antioxidative status in the early phase of acute myocardial infarction and after PTCA.

Materials and Method

The study was performed in the Ataturk University Cardiology Department according to the criteria of the Helsinki Declaration.

Introduction

Today, the prevalence of cardiovascular diseases has been increasing worldwide. Coronary artery disease constitutes the majority of these cardiovascular diseases. The rupture of luminal plaques, which consist of cholesterol and lipids, leads to the

The study protocol was confirmed by the Ataturk University Medical Faculty Ethics Committee. Informed and signed consent was obtained from all patients.

Patients with Acute Myocardial Infarction (AMI) admitted to our clinic were included in the study. Inclusion criteria were AMI symptoms for more than 30 minutes, admission six hours after onset of symptoms, >0.1mv ST segment elevation in two or more contiguous ECG leads, TIMI 0 flow with a total occlusion in coronary angiography and provision of TIMI 3 flow after PTCA. Exclusion criteria were lack of patient's consent for study, chronic renal failure and any chronic inflammatory disease. 25 patients with Acute Myocardial Infarction (AMI) treated successfully with PTCA and 25 healthy volunteers were included in the study.

Medical therapy in patients with the diagnosis of AMI was performed in accordance with the American College of Cardiology/American Heart Association guidelines. All patients were given 300 mg acetylsalicylic acid (ASA) and 5000 IU heparin before PTCA. A weight (kg) adjusted tirofiban bolus was given to patients with thrombosed lesions during PTCA and the drug was continued at a maintenance dose after PTCA. In light of the guidelines, acetylsalicylic acid, clopidogrel, beta blockers (metoprolol, nebivolol), antihyperlipidemic agents and angiotensin converting enzyme inhibitors were added to therapy. Blood samples were collected into vacuum tubes with EDTA at admission (0 hour), sixth and 24th hour. Only one blood sample was taken from the control group.

Biochemical measurements

Erythrocytes were sedimented by the centrifuge method and haemolysed with deionised water. The haemoglobin values of patients and the control group were determined by Gen-S haematology analysis device. The samples were kept at -80°C until biochemical analyses were performed (up to 2 weeks). Total Superoxide Scavenger Activity (TSSA) and Non-enzymatic Superoxide Scavenger Activity (NSSA) measurements were performed before and after the addition of trichloroacetic acid (TCA, 20%) into samples. TSSA measurement was performed first, then NSSA measurement was conducted with the removal of proteins by centrifugation of the sample added TCA 20% solution, at 5000*g for 30 minutes. Superoxide dismutase (SOD) activity was calculated by subtracting NSSA from TSSA. The measurement of malonyldialdehyde (MDA), which is an important marker of oxidative stress, is achieved by spectrophotometric analysis of thiobarbituric acid reactive products (TBARS). Glutathione (GSH) is detected with the Beutler method [5]. The glutathione peroxidase activity was determined spectrophotometrically [6]. The 8-hydroxydeoxyguanosine (8-OHdg) levels were measured by ELISA.

Statistical analysis

Data was analysed with SPSS 20.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA). A Paired-Samples T test was used to compare parametric variables between the patient and control groups and Chi-square test for categorical variables. The Kolmogorov-Smirnov test was used to determine whether the variables were normally distributed. The continuous

variables were expressed as mean $\pm$ standard deviation categorical data was expressed as percent (%). A p value <0.05 was considered to be statistically significant.

Results

Eighty-eight percent of 25 patients and 84% of 25 healthy volunteers were males. The mean age among both groups was similar (55 $\pm$ 5, 52 $\pm$ 6; NS). Smokers constituted 48% of the patient group and 43% of the control group and there were no significant difference. Diabetes mellitus was found to be 4% in patient groups. Hypertension was detected in 16% of patients. In healthy volunteers there were no hypertension or diabetes mellitus patients. Anterior and inferior myocardial infarction constituted 52% and 48% of patients, respectively. The patients total cholesterol, high density lipoprotein and low density lipoprotein levels were significantly higher than the control group. All of the patients were given acetylsalicylic acid, clopidogrel, enoxaparine and statins. Beta-blockers and ACE inhibitors were used if required in 66% and 38% of patients, respectively, due to their rhythm and blood pressure values. Nitrates could be used in 23% of patients. Tirofiban was given to 95% of patients and started during PTCA with a bolus dose and a maintenance infusion was continued for 24 hours. The health status, general characteristics of subjects included in the study and the therapy given for patients are shown in Table 1.

No significant increase was observed in malonylaldehyde levels between patients (3.99 $\pm$ 1.14, 4.05 $\pm$ 1.35, 3.62 $\pm$ 1.11nmol/g Hb, p<0.05) and the control group (4.76 $\pm$ 1.4nmol/g Hb) at 0, 6th and 24th hours. 8-OHdg levels increased in higher significance at 0 and 6th hours compared to the control group (2.52 $\pm$ 1.27,

Table 1: The health status, general characteristics of subjects included in the study and the therapy given for patients.

	AMI group (n=25)	Control group (n=25)	P value
Age	55 $\pm$ 5	52 $\pm$ 6	0.41
Male (%)	88	84	0.27
Hypertension (%)	16	-	-
DiabetesMellitus (%)	4	-	-
CurrentSmoker (%)	48	43	0.07
PriorInfarction (%)	0	0	-
Total Cholesterol (mg%)	205 $\pm$ 45	154 $\pm$ 39	<0.001
LDL cholesterol (mg/dl)	150 $\pm$ 28	126 $\pm$ 43	<0.05
HDL cholesterol (mg/dl)	32 $\pm$ 10	41 $\pm$ 12	<0.001
Aspirin (%)	100	-	-
ACE (%)	38	-	-
β -blocker (%)	66	-	-
Clopidogrel (%)	100	-	-
Tirofiban (%)	95	-	-
Enoxiparine (%)	100	-	-
Statin (%)	100	-	-
Nitrate (%)	23	-	-

2.56±1.36 10⁶dG, p<0.001) and there was a significant increase at the 24th hour (2.50±1.63 10⁶ dG, p=0.001). The levels of antioxidants like NSSA, TSSA, NO, SOD were significantly higher in the patient group than the control group at 0, 6th and 24th hours. Compared to the control group, GPX and GSH levels were found to be higher in the patient group without higher statistical significance. There was no significant difference in levels of oxidants and antioxidants between 0 and 6th, 0 and 24th and 6th and 24th hours. The concentrations of oxidants and antioxidants at 0, 6th and 24th hours are shown in Table 2.

Discussion

Myocardial ischemia can be detected in many conditions like atherosclerosis, thromboembolism, coronary artery bypass graft operation and percutaneous transluminal angioplasty. The common problem in all of these conditions is the inability to supply oxygen and substrates necessary for myocardial metabolism. Free oxygen radicals are formed by reduction of oxygen and under normal conditions they are removed by the body's scavenger systems. During myocardial ischemia there is an excessive production of these free oxygen radicals and the oxidative-antioxidative balance is impaired in favour of oxidation [7]. Our study partially supports the findings in previous studies related to oxidative and antioxidative state in patients with acute myocardial infarction. However, it provides some new data about the situation after PTCA. MDA is a ketoaldehyde found in great amounts after lipid peroxidation, assumed to be a marker of oxidative injury because of its relationship with lipoproteins. Cavalca V, et al. [8] detected a significant increase in MDA levels in patients with coronary artery disease compared to the control group. Pucheu S, et al. [9] reported that MDA is a useful marker in evaluation of oxidative stress after reperfusion of ischemic myocardium and they detected a significant increase in MDA levels in patients treated by intravenous thrombolytics compared to those without this therapy. In our study we found that there was no significant increase in MDA levels compared to the control group. However, a significant decrease was found in the patient group. This situation can be due to the effects of statin therapy started at admission and because of its elimination from the system after coronary reperfusion provided by PTCA. 8-OHdG levels give us information about oxidative DNA damage and the activity of DNA repair mechanisms. The removal of

lactic acid produced by myocardial metabolism decreases after ischemia, resulting in acidosis. The activity of DNA repair enzymes decreases as a result of acidosis. Reperfusion achieved by PTCA causes an increase in the activity of DNA repair enzymes. In rat studies 8-hydroxydeoxyguanosine levels were found to increase in infarcted regions [10]. Nagayoshi Y, et al. [11] showed that in patients with acute myocardial infarction, 8-OHdG levels were high at the beginning, reached their peak at the fourth hour after reperfusion and started to decrease at the 24th hour. In a study by Solen H, et al. [12] it was reported that in patients with acute myocardial infarction, the 8-OHdG levels were high in the first day and showed a sharp decline at the third day in patients treated by PTCA. Toth-Zsamboski E, et al. [13] found that 8-OHdG increased rapidly after PTCA and returned to normal levels after 96 hours. In our study, we detected that at the beginning and sixth hour after PTCA, 8-OHdG levels were significantly higher in the patient group than the control subjects, however, at the 24th hour, 8-OHdG levels were higher again without higher statistical significance and started to decrease. These results are similar to previous studies and 8-OHdG levels can provide information about the success of PTCA in the early phase, although further studies are needed. This early cleaning from blood could be a cause of tirofiban usage.

Reperfusion therapy must be conducted as earlier as possible after myocardial infarction in order to prevent ischemic injury. However, reperfusion is known to cause additional myocardial damage. A hypothesis proposes that sudden increase in oxygen metabolism after reperfusion causes a kind of insufficiency of anti-oxidation during myocardial infarction in patients with deficient defence systems [14]. Nevertheless, unlike some previous studies and hypothesis, in our study we found that antioxidant levels did not decrease, furthermore some markers increased significantly. Dusinovic S, et al. [15] did not find any significant change in the activity of SOD and GPX at the beginning and 1, 3, 6 and 24 hours of thrombolytic therapy in AMI patients. Simic D, et al. [16] reported that in patients with acute myocardial infarction after successful reperfusion, a significant increase was detected in SOD and GPX activities at the first hour, which continued up to the third day. Tomado H, et al. [17] showed that in AMI patients admitted 6 hours after symptom onset who had undergone PTCA, SOD activity started to increase at the first hour

Table 2: The concentrations of oxidants and antioxidants at 0, 6th and 24th hour in the control and AMI group.

Control Group		AMI Group		
		0	6	24
MDA (nmol/g Hb)	4.76±1.4	3.99±1.14*	4.05±1.35*	3.62±1.11*
8-OHdG (10 ⁶ dG)	1.25±0.51	2.52±1.27¥	2.56±1.36¥	2.50±1.63 µ
NSSA (U/mg Hb)	2.44±0.33	7.80±2.37¥	7.79±2.65¥	8.10±2.31¥
TSSA (U/mg Hb)	20.78±4.7	64.90±18.86¥	65.38±21.49¥	64.34±17.67¥
GPX (U/L)	42.4±18.3	50.96±30.60*	57.73±30.16*	54.21±23.59*
SOD (U/mg Hb)	989.6±156.4	1545.80±484.5¥	1555.30±518.8¥	1981.9±1977.1¥
GSH (nmol /L)	39.8±18.5	41.68±12.57*	46.59±13.28*	41.52±18.39*

Difference between the control *: p<0.05; ¥: p<0.001; µ: p=0.001

Difference between 0-6, 0-24, 6-24 were not significant.

and returned to its baseline levels at the 8th hour. In a study by Kharb S [18], it was shown that GSH levels were lower and MDA levels were higher in AMI patients treated by thrombolytics. Supporting our results, Muzakova V, et al. [19] showed that GPX activity reached its maximum concentration at the 90th minute and returned to its baseline value at the 18th hour after PTCA. In our study we found that SOD levels persisted to be significantly higher at the 24th hour also, GPX and GSH levels without reaching statistical significance were higher in the patients compared to the control group. GPX and GSH increase could be a front line defence against oxidative damage and is rapidly depleted. The down regulation of GPX and rapid elimination of GSH can explain why their concentrations did not reach higher significant levels and elevations in their concentrations could not be detected. In a higher rate usage of tirofiban could be helped to rapid reperfusion at the micro vascular level and it could accelerate the elimination of these front line defences.

Conclusion

Successful reperfusion provides rapid clearance of free oxygen radicals and supplies oxygen for myocardium under oxidative stress. In contrast to the previous studies, reperfusion obtained by drugs especially with Tirofiban and PTCA during AMI according to the guidelines, affects oxidation-anti-oxidation balance positively and diminishes the oxidative stress and shows advances in reperfusion therapy. The oxidative and anti oxidative parameters (8-OHdG level and SOD activity, etc.) defined in the study can be used to evaluate the success of PTCA, although further large studies are needed.

Limitations of the Study

The small number of patients in the study is a limitation. Another limitation is that at the time when they were included in the study, the patients were under drug therapy (beta blockers, acetylsalicylic acid, angiotensin converting enzyme inhibitors and statins), which can affect the levels of oxidative and antioxidative stress markers. However, ethically we cannot delay drug administration.

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