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Circulating p53 and cytochrome c levels in acute myocardial infarction patients

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Abstract *Background* Apoptosis causes myocardiocyte loss during and after myocardial infarction. Therapeutic approaches designed to arrest apoptosis would be a significant new development in the recovery of acute myocardial infarction (AMI). In order to examine apoptotic markers in the circulation, serum levels of p53 and cytochrome c were assessed in patients with AMI. *Methods* Blood samples were taken on admission (before initiation of therapy) and on the 3rd and 7th days of hospitalization. Serum levels of p53 and cytochrome c were measured by enzyme-linked immunassay. *Results* The serum level of p53 was higher in AMI patients on admission compared to the control group. A time-dependent decrease was observed in the serum level of p53, but there was no significant change in the serum level of cytochrome c during therapy. *Conclusions* p53, but not cytochrome c, appears to have potential as a biomarker for reporting on apoptosis following myocardial infarction.

Keywords Apoptosis · p53 · Cytochrome c · Acute myocardial infarction

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Introduction

Programmed cell death, or apoptosis, is a process for the orderly disposal of unwanted cells, and it has been implicated in a wide variety of physiological and pathological events. Apoptosis during the both acute and chronic stages of myocardial infarction has been demonstrated by animal and human studies [1–3]. Apoptosis has been implicated in unfavorable remodeling of the left ventricle characterized by ventricular dilation and decreased cardiac performance [4–6]. Reactive oxygen species (ROS) produced during prolong ischemia and reperfusion lead to an increase in the formation of DNA strand breaks, which in turn activates p53 gene. p53 protein is a transcription factor that is responsible for the regulation of cellular response to damage. p53 protein acts to induce cell cycle arrest or apoptosis in response to DNA damage [7]. Apoptosis can be initiated via endogenous death receptors (Fas). Alternatively, it can be initiated by mitochondria, which release cytochrome c into the cytoplasm by the mediation of some cytosolic proteins as a response to extrinsic stimuli, such as oxidative insults [8, 9]. In the cytoplasm, cytochrome c binds to a pro-apoptotic protein to form a complex that activates caspases. Activated caspases leads to activation of endonucleases, which in turn degredate the DNA [10, 11]. Cytochrome c released from apoptotic cells is an important marker of apoptosis, and its serum level has been determined in various studies [12–14].

The most reliable serum maker of apoptosis is soluble Fas ligand, which is the ligand of Fas. Its increased level in myocardial infarction has been shown previously [15]. We have scanned the apoptotic proteins that have not been examined in acute myocardial infarction (AMI) so far and noticed that data about serum levels of p53 and

cytochrome c are highly limited. The purpose of this study was to examine p53 and cytochrome c levels as markers of apoptosis in the serum of patients with AMI and to examine the relations between p53 and cytochrome c levels and other cardiac markers.

Materials and methods

The study subjects consisted of 28 consecutive patients (20 male and 8 female) with AMI who were admitted to the Cerrahpasa Medical Faculty Hospital's Cardiology Department, Coronary Care Unit. Institutional Ethics Committee approval was given in accordance with the principles of the Declaration of Helsinki, and informed consent was obtained from the relatives of each patient. The inclusion criteria were age >18 years, typical chest pain lasting >20 min but <12 h, acute ST-segment elevation myocardial infarction (STEMI) and acute non-ST-segment elevation myocardial infarction (NSTEMI) diagnosed according to the European Society of Cardiology and American College of Cardiology criteria [16]. Twenty-two of the patients were diagnosed with acute STEMI and six with NSTEMI. We excluded patients with renal insufficiency (serum creatinine $\geq$1.5 mg/dl), history of heart failure, acute and/or chronic inflammatory disease, thyroid disorder or cancer and who were taking antioxidant vitamins. Blood samples were taken on admission (before initiation of therapy), and on the 3rd and 7th days of hospitalization in all patients with both STEMI and NSTEMI. 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 the door-to-baloon time was <90 min; otherwise, thrombolytic therapy was preferred according to the recommendations of the ACC/AHA guidelines for patients with STEMI. Patients with NSTEMI did not undergo coronary intervention therapy during the hospitalization period. The control group consisted of 30 age-matched, normotensive, normolipidemic healthy volunteers (10 female, 20 male; mean age: 60 ± 10). None of the subjects were taking antioxidant vitamins.

Blood sampling and measurement of p53 and cytochrome c

Five milliliters of venous blood samples was taken from controls and AMI patients on admission and on the 3rd and 7th days of hospitalization. Following centrifugation at 3,000 $\times$ g for 10 min, serum was removed and kept at -80°C until the time of analysis. A serum level of p53 and cytochrome c were measured with competitive ELISA kits from Bender MedSystems (catolog no.: BMS256) and Chemicon International (catolog no.: APT200), respectively. The ELISAs for the p53 and cytochrome c were performed according to the manufacturer's instructions.

Total cholesterol, HDL and LDL cholesterol levels were measured by spectophotometric methods. Total CK, CK-MB and troponin I levels were determined by chemiluminescent immunometric assay, and the CRP level was measured by nephelometric assay.

Statistical analysis

Measured parameters are expressed as mean $\pm$ SD. Abnormally distributed data in the control group and patient group on the admission were compared by non-parametric Mann–Whitney U test. In the patient group, time-dependent changes in the measured parameters were analyzed by one-way ANOVA with Tamhane test. When the patient group was divided into two subgroups according to the type of reperfusion therapy, i.e., primary PTCA and thrombolytic therapy, comparisons between those groups were examined by Mann–Whitney U test. Differences between groups were considered significant at $P < 0.05$. Spearman correlation coefficient was used for correlation analysis.

Results

For 8 patients, primary PTCA was performed, and 14 patients were given thrombolytic therapy. Demographic data of the patients, clinical parameters, angiography and laboratory test results are shown in Table 1. Serum level of p53 was at a higher level in AMI patients on admission compared to the control group. A time-dependent decrease was observed in the serum level of p53 (Fig. 1). The decrease was significantly different between admission and the 7th day of the therapy. Although the p53 level on the 3rd day of the therapy was lower than those on admission, it did not reach a statistically significant level. The serum level of cytochrome c in AMI patients at admission was not significantly different from those of the control group, and there was no significant change in the serum level of cytochrome c during the therapy (Fig. 2). When the patient group was divided into subgroups in respect to the type of reperfusion therapy, no significant difference was found between the primary PTCA group and thrombolytic therapy group for either the serum level of p53 or of cytochrome c. No significant correlation was determined between the serum level of p53 or cytochrome c and CRP, CK, CK-MB and troponin I.

Table 1 Patient demographics, clinical parameters, angiography and laboratory test results at admission

AMI group (*n*:28)	
Demographic data	
Age (years)	60 ± 10
Patients with hypertension	14
Patients with type 2 diabetes mellitus	8
Obesity (BMI ≥30 kg/cm^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	
One-vessel	9
Two-vessel	3
Three-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–>100)

Data are expressed in numbers, or in medians and interquartile ranges, *ACE* angiotensin-converting enzyme, *CAD* coronary artery disease, *CPK* creatine phosphokinase

Discussion

Apoptosis and necrosis both cause to myocardiocyte loss; however, while apoptosis leads to silent but persistent loss, necrosis is associated with abrupt onset and clinical manifestation associated with secondary inflammatory phenomena [5]. Balance between apoptosis and primary necrosis depends on available energy levels, because completion of apoptosis needs adequate ATP [17]. Myocardial apoptosis peaks at 4–12 h in AMI and is persistently demonstrable up to 10 days [18, 19]. Olivetti et al.[18] showed that myocardial apoptosis is present at post-mortem examination in hearts of patients who died within 10 days after AMI. Various studies have determined the presence of apoptosis in end-stage heart failure [20–22] and, in particular, in post-infarction left ventricular (LV) remodeling [6, 23, 24]. Saraste et al. [22] showed an increase in myocardial apoptosis in cases of end-stage ischemic cardiomyopathy versus controls and determined that the apoptotic rate correlated with the clinical severity of heart failure. Baldi et al. [6] have examined the regional occurrence of myocardial apoptosis at the site of recent infarction versus remote unaffected areas at the time of autopsy up to 60 days after AMI. They reported that persistent myocardiocyte loss still occurs during the subacute phases of AMI, and there is a strong correlation between the apoptotic rate and macroscopic signs of LV remodeling. Post-infarction LV remodeling consists of progressive chamber dilatation, wall thinning and systolic/ diastolic dysfunction. LV remodeling is associated with unfavorable hemodynamic performance and adverse clinical outcomes during long-term follow-up, such as an increasing rate of symptomatic heart failure and sudden cardiac death [25].

To date, commonly, the presence of apoptosis in AMI has been shown by in situ end labeling of DNA fragmentation technique (TUNEL) or activated caspase 3 in hearts explanted ex vivo from patients.

After the confirmation of the role of apoptosis in AMI by those previous studies, the primary goal of future investigations should be the examination of apoptotic markers in the peripheral circulation during and after AMI. We have hypothesized that an increased apoptotic rate in AMI may reflect the peripheral circulation. The serum level of p53 and cytochrome c may reach a detectible level, and serum levels of those markers may be reliable markers for AMI. If the serum levels of p53 and cytochrome c are reliable markers for AMI, therapeutic approaches designed to arrest apoptosis would be a significant new development in the recovery of AMI. To the best of our knowledge, this is the first study examining the serum levels of p53 and

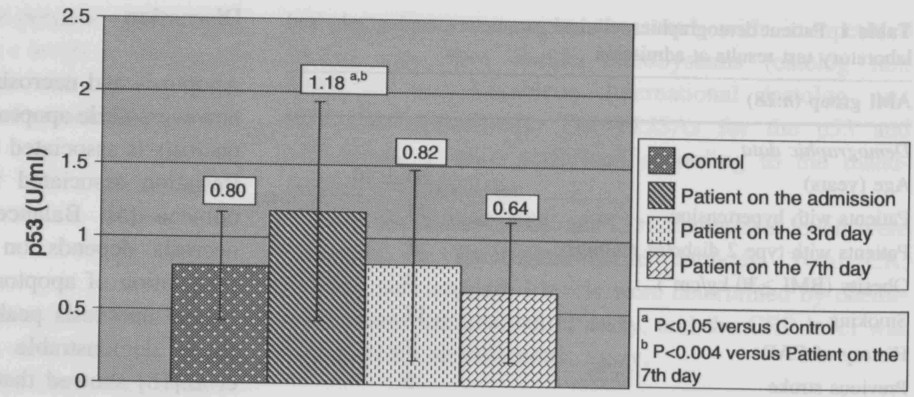

Fig. 1 Serum level of p53 in the control and AMI patient groups

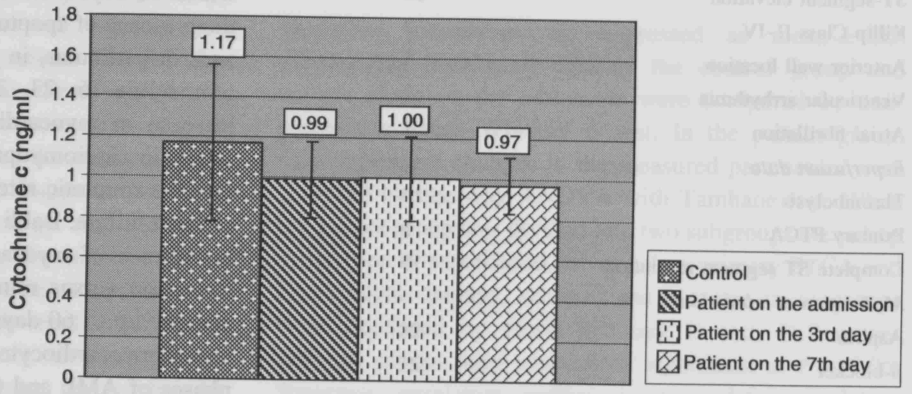

Fig. 2 Serum level of cytochrome c in the control and AMI patient groups

cytochrome c in patients with AMI. There is an animal study indicating that p53 expression can be increased during the first 3 days following reperfusion [26], but clinical data for the serum level of p53 is very poor. Kolomecki et al. [27] have measured p53 levels in the serum of patients with benign and malignant primary follicular thyroid tumors. In the present study, the serum level of p53 was higher in AMI patients on admission compared to the control group, and a time-dependent decrease was observed. The p53 level increases as a response to DNA damage; therefore, a decrease in the serum level of p53 may be attributed to overcoming a damaging event.

The circulating level of cytochrome c has been determined in a few studies so far. It has been measured by Ben-Ari et al. [13] in patients with liver disease, by Adachi et al. [28] in patients with systemic inflammatory response syndrome, and by Barczyk et al. [29] in cancer patients receiving therapy. In our study, there was no significant difference between controls and AMI patients on admission, and no change was observed in the serum level of cytochrome c during therapy. However, this finding does not display the absence of apoptosis. As a result of its consumption in the cytoplasm by binding to some cytosolic proteins to form caspase-3 activation, cytochrome c may not be elevated in the serum. Alternatively, intervals of the measurement (on the admission, 3rd and 7th days) may be too large to catch an increase in the serum cytochrome c level.

This was a preliminary study with a limited number of parameters and a limited number of subjects. Data reveal that cytochrome c is not a proper indicator for demonstration of cardiomyocyte apoptosis in the peripheral circulation of AMI patients. More appropriate parameters (for example, activated caspase 3) should be examined in future studies. In conclusion, p53, but not cytochrome c, appears to have the potential to be a biomarker for reporting on apoptosis following myocardial infarction.

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