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Acute Myocardial Infarctıon Ischemia of The Erythtrocyte Oxidative Stress In The Role Of Nitric Oxide

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ABSTRACT

Acute myocardial infarction (AMI) patients' that ischemia / reperfusion injury to, the information is controversial anti-oxidant enzymes. Thrombolytic therapy in studies that compared the values before and after treatment, erythrocyte antioxidant activity has been reported that an increased-decreased or remained unchanged. Acute inflammatory events, nitric oxide (NO) as a paradox of both protective and damaging effect is remarkable. In our study, ischemia during AMI because of the variable state parameters of oxidative stress malondialdehyde (MDA) and superoxide dismutase (SOD) and NO in plasma and red blood cell measurements, and the latter with the evaluation was to determine erythrocyte has acted as an antioxidant. This study in 50 patients with a diagnosis of AMI and 30 healthy adult human, a total of 80 persons were taken into consideration. After obtaining blood samples, plasma and red blood cell pellet samples stored at -80 until analysis. Thiobarbituric acid method of MDA, SOD activity and NO levels of nitroblue tetrazolium method, the modified cadmium reduction method, were measured spectrophotometrically. Acute MI group than the control group, plasma MDA and NO values were significantly higher ($p < 0.005$), whereas erythrocyte MDA levels were similar in the control group. On the other hand, erythrocyte SOD activity and NO levels in the AMI group, low in the control group ($p < 0.005$) were found to be. NO levels of patients diagnosed with AMI and the significant decrease in erythrocyte SOD, erythrocyte SOD, catalase (CAT) and glutathione peroxidase (GPx), which consists of the first cellular line of defense may play a role also suggests that NO. AMI showed a significant increase in plasma MDA levels, erythrocyte MDA levels, while not exhibiting, supports this opinion.

Key words: Myocardial infarction, erythrocyte, malondialdehyde, nitric oxide, superoxide dismutase.

INTRODUCTION

Acute Myocardial Infarction (AMI), inadequate tissue perfusion due to irreversible myocardial cell damage and necrosis (1). AMI by the beginning of the development of atherosclerosis, one of the important factors in the nitric oxide (NO). Atherosclerosis, vascular endothelial function and NO production is reduced causing a violation (2). Animal models of study, NO inhibition can increase the development of atherogenesis is shown (3,4). In the absence of nitric oxide, increasing smooth muscle cell activation and proliferation of cells included to provide up to atherosclerosis (5). Vessels supplying the heart muscle, changes in atherosclerotic vascular thrombosis and acute MI is caused by obstruction of the lumen (5). NO synthesis is organized with the enzyme NO synthase (NOS). Structural NOS vascular endothelium, neurons and platelets, neuronal nitric oxide synthase (nNOS) found to neurons, endothelial nitric oxide synthase (eNOS) is located in the endothelial cells. Basal levels of the various organ systems necessary for the structural NOS, while the structural effect of NOS generated NO is required for maintenance of normal physiological events (6). On the other hand, the effect of iNOS produced high concentrations of NO, improves endothelial and vascular damage (7). This reality, many researchers in a review of the complexity of the causes of this issue leads to many new investigations.

SOD an enzyme naturally found in the mitochondria within the cell and the activity of this enzyme activity is high in tissues that use oxygen to tissue pO 2 (partial pressure of oxygen) increases with the increase.

Physiological function of the enzyme using oxygen to protect cells against the harmful effects of superoxide free radicals. This effect, which is less reactive superoxide radicals of hydroxy peroxide performs rotating form (8.9). This increase of free oxygen radicals after ischemia as an enzymatic defense response increasing of SOD, but the amount of SOD is reduced and the amount of superoxide radical, as an alternative medium in this case consists of peroxynitrite, NO reacts with superoxide and consequently may decrease the effect NO's vasodilator. A strong oxidant is considered to be a long half-life the resulting peroxynitrite. The reduction of hydrogen peroxide shows an increase in hydroxyl radical production case of missing by CAT. Free radicals especially hydroxyl radical, which the most reactive radical plays an important role in lipid of the peroxcidation and hemolysis in erythrocytes may also contribute.

SOD is an enzyme naturally found in the mitochondria within the cell, this enzyme activity is high in tissues that use oxygen and . Physiological function of the enzyme using oxygen protect cells against the harmful effects of superoxide free radicals. This effect, which is less reactive superoxide radicals of hydroxy peroxide performs rotating form (8.9n in). The powerful oxidant peroxynitrite formed considered to be a long half-life. Lipid peroxidation occurs as a result of the MDA a marker of lipid peroxides (10).

In this study, the period of ischemia in patients AMI MDA, SOD and NO in plasma and red blood cell parameters and evaluation of a combination of measurements with two-way effect of NO in red blood cells was to determine in how you behaved.

EXPERİMENTAL SECTİON

Study population

This study in 50 patients with a diagnosis of AMI and 30 healthy adult human, a total of 80 persons were taken into consideration.

This study has been included in the total of 80 people of whom the hospital's emergency department with complaints of chest pain and 50 adult patients diagnosed with AMI and 30 healthy adults including control. The diagnosis of acute MI was reached based on the following criteria: (a) typically chest pain more than 30 minutes and not relieved by sublingual glyceryl trinitrate; (b) more than 0.1 mV ST segment elevation in two anatomically contiguous leads or more than 0.2 mV in leads V1 and V2; and (c) elevation of levels of MB fraction of creatine kinase (CK-MB) or troponin-I at least three times the upper reference limit. The study began after approval was obtained from the local Ethics Committee and informed consent from all patients were included.

Those with a history of thrombolytic therapy, those who apply to the hospital 12 hours after the start of chest pain, those who had major surgery and rheumatic, oncological, ESRD, those with infectious disease not included.

Study protocol

Blood samples of patients diagnosed with acute myocardial infarction in the emergency room within the first 30 minutes when, in the control group were taken after 12 hours of fasting. After clotting the samples were subjected to the process of centrifugation time 5 min at 3000 circulation, then stored at -80 until analysis plasma samples were obtained.

After getting the rest of the blood plasma at the bottom of the tube-shaped elements, the inner walls of the tube by the method of leakage was diluted by adding approximately the same volume of saline. Slowly lower the upper tubes, after being closed with the parafilm, 2000 circulation süpernatanlar was centrifuged for 10 minutes. Again on the same tube at approximately the same volume, the same procedure was repeated by adding saline. Erythrocyte washing process by applying 3 times, pure red cell pellet was obtained.

Hemolysis, obtained from the erythrocyte pellet in 0.5 ml was achieved based on the addition of 2.0 ml of cold distilled water. and stored at -70 until analysis hemolizatlar. Hemolysis, MDA and Hb analysis was performed. Later this hemolysis sample, taken in 500µL (250 mL) ile1500 mL (750µL) in cold distilled water, mixed with a glass tube. Obtained from this mixture, 1 ml and 3 / 5 was prepared by ethanol / chloroform mixture was transferred to a glass tube with 1 mL of a taking. Centrifuged at 4000 rpm for 30 minutes at the end of the upper phase (ethanol phase) was carefully collected and SOD analysed.

Blood sample analysis

MDA analysis, based on the principle of heat reaction of MDA with TBA Hammouda et al Thiobarbituric acid (TBA) method, were studied (11). Thiobarbituric acid reacting with an acid environment at 90 ° C, the MDA, is pink-colored chromogen. Chromogenic color intensity is proportional to the MDA environment, spectrophotometer at a wavelength of 532 nm absorbance is read against the blind by taking advantage of the value of the plasma MDA values in nmol / ml, respectively.

SOD activity analysis, Sun et al (12) according to the methods and Durak et al (13) was defined according to their modification. Detection method: xanthine / xanthine oxidase system produced by superoxide, nitro blue tetrazoliumu (NBT) reduction based on the principle of the superoxide radicals and NBT environment "by reducing color formation is good. This complex has a maximum value of 560 nm absorbance. This reduction, the enzyme does not come in the blue-purple color ,is composed of environment. When the environment is SOD, NBT reduction but

blue do not come in purple color and light color; depending on the activity of the enzyme and is composed of the amount.

Total nitrite (nitrite + nitrate) concentration of nitrate / nitrite colorimetric kit method (Cat. no. CM780001, Cayman Chemical Company, USA) were evaluated according to the reaction of end-to-read color spectrophotometrically at a wavelength of 540 nm, for plasma micromolar and for erythrocytes micromolar / grHb.

Statistical analysis

SPSS 13.0 package program was used for statistical analysis. Continuous variables were expressed as mean ± SD. Parametric Student's t-test was used to compare the data. Frequency and chi-square test was used to compare the rates in addition to p value of <0.05 was considered significant.

RESULTS AND DISCUSSION

Table1. Exculsion criterias were defined as following

Earlier the times those who had AMI,
AMI was diagnosed, but those who have received thrombolytic therapy,
Chest pain, those who apply to the hospital after 12 hours,
Those who spent the last 6 months, major surgery,
Rheumatic, oncological, and infectious disease,
Disease in ESRD.

Table 2: Cases of AMI and the control group MDA, SOD and NO parameters, AO ± SD values and the statistical results

Parameters	**AMI**	**Control**	**P**
Patients Invalid	50 person	30 person	
Plasma			
MDA (nmol / ml)	1.91 ± 0.59	1.37 ± 0.52	< 0.005
NO (µM/ L)	31.58 ± 9.51	21.06 ± 9.01	< 0.005
Erythrocyte			
MDA (nmol/gr Hb)	20.72 ± 9.96	17.64 ± 12.33	> 0.05
SOD (U/gr Hb)	1501.28 ± 919.93	2668.64 ± 379.96	< 0.005
NO (µM/ grHb)	0.45 ± 0.28	0.73 ± 0.37	< 0.005

Plasma MDA and NO values increasing were faund ($p<0.005$), but erythrocyte MDA value increase was faund insignificant. SOD activity in erythrocyte and NO levels were faund decreased ($p<0.005$) (Table 1).

DISCUSSION

The contribution of NO, which is a key role in the development of atherosclerosis is a general assumption in all cases. AMI in this situation is unknown, but the exact mechanism of NO reduction activity of NO in different phases of the path changes, not limited, making the reduction of NO is thought to be due, or the reduction of NO bioavailability. NO reduction in the synthesis of NO in the construction of the nitric oxide synthase (NOS) enzyme, substrate and cofactor deficiency originate, oxidative stress is thought to decrease the bioavailability of NO.

Synder et al (1992) and Murad et al (1999), their experimental animal models of NO in the progression of chronic inhibition of atherogenesis have shown increased (14). Collier et al is expressed in 1989 of the NO's decrease a role in the increase most important effect of NO inactivation of the by superoxide anion (15). The same time Vallance et al in 1992 are showed increased oxidation of LDL particles leading to the formation of a strong oxidant peroxynitrite (16). Inadequate tissue perfusion caused by decreased blood flow as a result of prolonged

ischemia, can increase NO production by eNOS activation. In the region as a result of ischemic cell infiltration of leukocytes de-granulation with the increase in free radicals, may lead to an increase in the MDA. Indeed, cases of AMI in our study, the increase in plasma MDA, reflects this situation.

Hypokinetic, the other with an expression of ischemic hypoxia, decreased blood flow is provisioned with sufficient oxygen, depending on lung tissues from the blood draw in more oxygen and this causes the RBCs to hypoxia. The reduction in erythrocytes SOD activity inactivation of NO with excess superoxide production. NO as a radical with low reactivity, especially the antioxidant effect of lipid radicals may react. Indeed this study cases of AMI in the control group was insignificant increase in erythrocyte MDA level, SOD activity and NO level significantly decreased SOD enzyme. The latter enters into a reaction of lipid radicals and lipid peroxidation suggests restrain.

The superoxide reaction of NO's in erythrocytes of the SOD confirmed the role of antioxidants. The resulting peroxynitrite, plasma, red blood cells through the physiological environment nitrosyl-tiol, nitrosyl-glukoz nitrosyl-albumine and intermediates such as the vasodilator effect of NO and to the alternates.

Our study had a significant increase in plasma NO, this reflects the junction. In our study, decreased plasma NO level was increased by erythrocyte NO, it can be reflected. From this point study results; NO erythrocyte antioxidant activity in opposition to while the plasma the endothelium vasodilator activity in the foreground. Therefore due to increase in oxidant stress during ischemia AMI is a significant in plasma MDA and junction to this idea. Decreased red blood cell NO levels increase in plasma NO levels, RBCs may be related shift in plasma NO. This shift, as conceivable as a positive regulatory mechanism in addition to this, also believe that because of secondary reduction of red cell NO.

At the end of the reaction of nitric oxide superoksitle peroxynitrite (ONOO-), a substance that can lead to tissue damage and is very strong oxidative properties and has cytotoxic and inflammatory effects of nitric oxide is responsible. Pre-light, while AMI, AMI became evident during the hypoxic environment, the increase in blood peroxynitrite, endothelial dysfunction may play an important role.

On the coronary vessels ONOO - 's effect on isolated coronary artery preparations to examine URLs, a study of ONOO - in the endothelium-independent relaxation of coronary arteries was shown that (17). In the relaxation ONOO - caused by SOD ¬ is intended to overcome the rages with hemoglobin. So, this relaxant effect be explained by the formation of peroxynitrite to NO ¬ as well as returned.

Another study with the isolated perfused rat heart in ¬ ONOO - in rat coronary vascular bed has been shown to cause dose-dependent relaxation (18). In addition, this relaxation might have prevented ONOO-mediated vasodilatation of NO 's by oxyhemoglobin ¬ responses to cold floor.

CONCLUSION

In our study were determined meaningless increase in the erythrocyte MDA, but the significant decrease in SOD and NO levels are values. Taken together these two are determined, the first cellular defense line to protect erythrocytes SOD, CAT and GPx, such as free radical scavengers, nitric oxide has emerged as a result be included.

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