Reperfusion injury has also been attributed to production of large amounts of reactive oxygen species (ROS). (3) Reperfusion followed by AMI results from the initial loss of blood flow and clearance of oxygen free radicals from the cellular environment. The first results from the initial loss of blood flow and clearance of oxygen free radicals from the cellular environment. The second results from the restoration of oxygenated blood flow. The tissue is subjected to reperfusion, which can lead to the formation of free radicals and reactive oxygen species. This can result in oxidative stress and potential damage to cellular components. The body has mechanisms to preserve energy levels and prevent injury. (1) These mechanisms include the production of antioxidants, such as total thiols, which play a major role along with other antioxidants in preserving cellular integrity. Increased generation of ROS may be responsible, at least in part, for irreversible peroxidative damages to myocardial tissue, which may persist for months following thrombolytic therapy. (4) Among the ROS, malondialdehyde (MDA), a lipid peroxidation end product, is considered as one of the markers of cell membrane damage. (5) The major antioxidant in the body fluids is the cystein-SH bound to protein, majority of it found on albumin, and glutathione (GSH). These –SH groups of cell membrane help in ameliorating the lipid peroxidative effects of ROS. (6,7)

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In the current work we have measured malondialdehyde (MDA), total thiols, total CK, CK-MB and AST in ECG proven acute myocardial infarction (AMI) patients immediately after admission and 24 hours after administration of thrombolytic agent streptokinase, and in healthy controls. Methods: Blood samples from 44 AMI patients and 25 age and sex matched healthy controls were obtained and analyzed for MDA, total thiols using spectrophotometric methods and cardiac enzymes total CK, CK-MB and AST using automated analyzer. Results: We have found significant increase in MDA, CPK, CK-MB, AST (p<0.001) and significant decrease in total thiols (p<0.001) in AMI patients after thrombolytic therapy compared to values at admission, and healthy controls. MDA correlated negatively with total thiols (τ = 0.333, p<0.05) and positively with CK-MB (τ = 0.491, p<0.01) in AMI patients after thrombolytic therapy. Conclusions: Reperfusion following thrombolytic therapy increases reactive oxygen species with concomitant decrease in antioxidant total thiols.

Key Words: MDA; Total thiols; Myocardial infarction; Reperfusion injury

Abstract:

Background: Reactive oxygen species have been implicated in the pathogenesis of ischemic and reperfusion injury. In the current work we have measured malondialdehyde (MDA), total thiols, total CK, CK-MB and AST in ECG proven acute myocardial infarction (AMI) patients immediately after admission and 24 hours after administration of thrombolytic agent streptokinase, and in healthy controls. Methods: Blood samples from 44 AMI patients and 25 age and sex matched healthy controls were obtained and analyzed for MDA, total thiols using spectrophotometric methods and cardiac enzymes total CK, CK-MB and AST using automated analyzer. Results: We have found significant increase in MDA, CPK, CK-MB, AST (p<0.001) and significant decrease in total thiols (p<0.001) in AMI patients after thrombolytic therapy compared to values at admission, and healthy controls. MDA correlated negatively with total thiols (τ = 0.333, p<0.05) and positively with CK-MB (τ = 0.491, p<0.01) in AMI patients after thrombolytic therapy. Conclusions: Reperfusion following thrombolytic therapy increases reactive oxygen species with concomitant decrease in antioxidant total thiols.

Key Words: MDA; Total thiols; Myocardial infarction; Reperfusion injury

Introduction:

In acute myocardial infarction (AMI), two distinct types of damage occur to the heart: ischemic injury and reperfusion injury. The first results from the initial loss of blood flow and the second upon the restoration of oxygenated blood flow. The heart can tolerate a brief exposure to ischemia as the inherent mechanisms to preserve energy levels prevent injury.(1) Reperfusion of ischemic myocardium is, therefore, a prerequisite for cellular survival. Reperfusion followed by AMI has several benefits such as significant increase in recovery of left ventricular functions.(2) Along with beneficial effects, reperfusion has also been attributed to production of large amounts of reactive oxygen species (ROS). (3)
vacutainers from the antecubital veins of AMI patients immediately after admission and 24 hours after initiation of thrombolytic therapy. Similarly, samples were also obtained from age and sex matched healthy controls. Total CK, CK-MB, AST, MDA, total thiols levels were measured in all the obtained samples after proper processing.

Reagents
Special chemicals like 5’ 5’ dithio-bis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), and standard MDA were obtained from sigma cheAMcals, St Louis, MO, USA. All other reagents were of analytical grade.

Biochemical Determinations
Measurement of cardiac enzymes:
Cardiac enzymes CPK, CK-MB and AST were measured using enzymatic assay using auto analyzer.(8-10)

Total thiol assay:
Reaction mixture contained 900 µL 2 mM Na₂ EDTA in 0.2 M Na₂HPO₄, 20 µL 10 mM DTNB in 0.2 M Na₂HPO₄ and 100 µL of serum. Reaction mixture was incubated at room temperature for 5 minutes; absorbance read at 412nm. Appropriate sample and reagent blanks were prepared simultaneously and the respective absorbance was noted. Corrected absorbance values were used to calculate serum total thiols using the molar extinction coefficient 1600 M⁻¹ cm⁻¹ and values expressed as µM. The calibration curve was produced using GSH dissolved in phosphate buffered saline.(11)

MDA assay:
Reaction mixture contained 1 mL 0.67% thiobarbituric acid (TBA), 500 µL 20% Tri carboxylic acid (TCA) and 100 µL serum. Incubated at 100°C for 20 minutes; centrifuge at 12,000rpm for 5 minutes. Absorbance of supernatant read at 532 nm. MDA was determined by using molar extinction coefficient 1.56 x 10⁵ M⁻¹ cm⁻¹ and values expressed as nM. (12)

Statistical Analysis
The results were expressed as mean ± standard error of mean (SEM). A p<0.05 was considered statistically significant. Statistical analysis was performed using the statistical package for social sciences (SPSS-16, Chicago, USA). One way analysis of variance (ANOVA) was used to compare the mean values in three groups, followed by multiple comparison post hoc tests. Pearson correlation was applied to correlate between the parameters.

Results:
As shown in Table 1 we have found significant increase in MDA, total CK, CK-MB, AST (p< 0.001) and significant decrease in total thiols (p<0.001) in AMI patients after thrombolytic therapy compared to values at admission and healthy controls. There is significant increase in levels of MDA (p<0.001) and decrease in total thiols (p<0.001) in AMI patients immediately after admission compared to controls. On applying Pearson’s correlation total thiol levels in serum correlated negatively with MDA (r = -0.333, p<0.05) (Figure 1). As depicted in Figure 2, there was significant positive correlation between CK-MB and MDA (r = 0.491, p<0.001).

Table 1: Total thiols, MDA, Creatinine kinase, CK-MB and AST levels in healthy controls and myocardial infarction patients at admission and 24 hours after streptokinase therapy (Values expressed in mean ± SD).

<table>
<thead>
<tr>
<th>Tests</th>
<th>Controls (n = 25)</th>
<th>AMI Cases (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On admission</td>
<td>24 hours after Streptokinase</td>
</tr>
<tr>
<td>Creatinine Kinase (U/L)</td>
<td>108 ± 27</td>
<td>188 ± 125*</td>
</tr>
<tr>
<td>CK-MB (U/L)</td>
<td>17 ± 2</td>
<td>38±22**</td>
</tr>
<tr>
<td>AST(U/L)</td>
<td>24 ± 5</td>
<td>40±38**</td>
</tr>
<tr>
<td>MDA (nmoles/L)</td>
<td>215 ± 51</td>
<td>362±53**</td>
</tr>
<tr>
<td>Total thiols (µmoles/L)</td>
<td>384 ± 70</td>
<td>306± 24*</td>
</tr>
</tbody>
</table>

*p<0.001 compared to healthy controls; *p<0.001 compared to AMI on admission

Figure 1: Correlation between MDA and Total thiols in AMI patients 24 hours after thrombolytic therapy
Discussion:
We have found increase in the MDA levels immediately after myocardial infarction compared to healthy controls. This may be due to oxidative stress induced by acute ischemic injury and similar observation was published by many previous authors.(6,13) We have also observed significant increase in MDA levels 24 hours after reperfusion, which possibly explained increased oxygen availability to ischemic site generating increased amount of ROS, thereby causing further injury to cell membrane. This may possibly explain the reperfusion injury observed by previous authors.(14,15)

We have also observed that increased MDA levels after reperfusion correlated positively with cardiac marker enzyme CK-MB, 24 hours after thrombolytic therapy, which may again explain the ROS mediated damage to myocyte membrane thereby increasing the release of cardiospecific marker CK-MB fraction.(1) Available literature explains that oxidative stress followed by reperfusion therapy may be due to variety of factors such as (1) Enhanced generation of ROS due to sudden massive increase in oxygen supply; (2) Reduced levels of antioxidants available, (3) Enhanced consumption, leakage or destruction of antioxidants, (4) Leakage of electrons from the disrupted mitochondrial electron transport chain and (5) Phagocytic recruitment and activation.(16) All of above effects together initiate lipid peroxidation in cell membrane, damage membrane proteins or cause DNA fragmentation. These processes may result in a loss of contractile function of cardiac myocyte and lead to severe myocardial cell damage collectively termed as reperfusion injury.(17)

We have found decrease in total thiols following reperfusion, which correlated negatively with MDA. The major part of thiols in plasma is derived from proteins, especially albumin, and they are susceptible to oxidation during reperfusion. Significant low level of total thiols during myocardial infarction and after reperfusion may indicate increased consumption of these thiol groups to neutralize increased levels of ROS in these states.(18) Decreased availability of thiols may also affect other biochemical function of the cell where thiols play an important role for the catalysis process.(19)

In conclusion the cardiac ischemia induces ROS production and subsequent reperfusion can result in toxic ROS overproduction with concomitant decrease in antioxidants.

References:

Figure 2: Correlation between MDA and CK-MB in AMI patients 24 hours after thrombolytic therapy