L-Arginine attenuates oxidative stress condition during cardiomyopathy

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Increased production of oxygen free radicals and decreased oxidant capacity occur in coronary artery diseases (CAD). This pro-oxidant shift in intracellular redox state may induce cell death by either direct cell membrane damage by lipid peroxidation or apoptosis through activation of transcription factors. These changes occur not only in cardiomyocytes, but also in cardiac sympathetic nerves, which are very sensitive to oxidative damage. Patients with heart failure encounter reduced peripheral blood flow at rest, during exercise and in response to endothelium-dependent vasodilators. Current treatments of cardiomyopathy, a degenerative condition of the myocardium frequently associated with heart failure have done little to enhance patient survival. Decreased myocardial contractility and altered regulation of peripheral circulation along with oxidative conditions are important contributors to the symptoms and prognosis of the disease process. Nitric oxide formed from L-arginine (2-amino-5 guanidinovaleric acid) metabolism in endothelial cells contributes to regulation of blood flow under these conditions. L-Arginine is the precursor of nitric oxide, an endogenous messenger molecule involved in a variety of endothelium-mediated physiological effects in the vascular system. In the present study, we investigated the effect of oral administration of L-arginine (3 g/day) on the intracellular redox status of the patients of ischemic cardiomyopathy aged 45-60 yrs. The enzymatic and non-enzymatic antioxidant parameters like superoxide dismutase, catalase, total thiols (TSH) and ascorbic acid along with pro-oxidant parameters, such as xanthine oxidase, as well as index of oxidative stress as protein carbonyl content and malondialdehyde (a marker of lipid peroxidation) were investigated in the plasma and RBC lysate. L-Arginine (3 g/day) administration was found to improve the levels of these parameters in the patients and regulate the blood flow, as evident by the improved blood pressure of the patients. Thus, it is inferred that L-arginine attenuates the oxidative stress conditions along with maintaining the blood pressure rate of patients suffering from cardiomyopathy.

Keywords: Cardiomyopathy, Oxidative stress, L-Arginine, Nitric oxide, Pro-oxidant shift.

Myocardial ischemia results from decreased oxygen availability to the myocardium. Insufficient oxygen supply fails to support oxidative phosphorylation. Anaerobically produced ATP in this situation is insufficient to meet the energy demand of the tissues. With continuing ischemia, the tissue levels of ATP fall and this initiates a series of events, especially oxidative stress conditions, which are deleterious to the endothelial cells. Oxidative stress associated with increased formation of reactive oxygen species (ROS) modifies phospholipids and proteins, leading to lipid per-oxidation and oxidation of thiol groups¹². These changes are considered to alter the membrane permeability and configuration.

Oxidative stress may result in cellular defects, including a depression in the sarcotrophal (SL) Ca²⁺ pump, ATPase and Na⁻-K⁺ ATPase activities, leading to decreased Ca²⁺ efflux and increased Ca²⁺ influx³ and thus inhibit Ca²⁺ sequestration from the cytoplasm in the cardiomyocytes³⁴. The oxidative stress-induced changes in sarcoplasmic reticulum (SR) Ca²⁺ and SL Na⁻-K⁺ pumps are not limited only to cardiomyocytes, but have also been observed in the coronary artery smooth muscle cells. The depression in Ca²⁺ regulatory mechanism by ROS ultimately results in intracellular Ca²⁺ overload and cell death⁵⁻⁶. On the other hand, an increase in Ca²⁺ during ischemia induces conversion of xanthine dehydrogenase to xanthine oxidase by selective proteolysis that generates more of superoxide radicals. The superoxide radical, though less toxic by itself, triggers the formation of other ROS including OH⁻, H₂O₂ and HOCl⁻. The hydroxyl radicals, in particular interact with lipids, proteins and nucleic acids, resulting in the loss of membrane integrity, structural and functional changes in enzymes, proteins

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Abbreviations: CAD, coronary artery diseases; CAT, catalase; GPX, glutathione peroxidase; GR, glutathione reductase; MDA, malondialdehyde; ROS, reactive oxygen species; SL, sarcotrophal; SR, sarcoplasmic reticulum; SOD, superoxide dismutase; XO, xanthine oxidase.
and genetic material\textsuperscript{7}. These deleterious effects result in the loss of heart contractile function and severe myocardial cell damage.

To counter the damaging effects of ROS, body has evolved certain defense mechanisms to deactivate/destroy them before they can cause damage. The oxidative damage will occur only in situations when defense mechanisms are deficient, made less active or production of ROS exceeds the capabilities of defense mechanism or a combination of all these. The first line of defense in the body are enzymatic free radical scavengers, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). SOD is considered to be the first line of defense against oxidative insult, as it efficiently converts oxygen-derived free radical to \( \text{H}_2\text{O}_2 \).\textsuperscript{7,8} The \( \text{H}_2\text{O}_2 \) is more toxic than oxygen-derived free radicals and is capable of producing most toxic OH radical. It has, therefore, to be removed very efficiently. CAT is the highly reactive enzyme in most of the tissues that converts toxic \( \text{H}_2\text{O}_2 \) to water. GPx is another enzyme that removes \( \text{H}_2\text{O}_2 \) at the expense of oxidation of glutathione and is almost equally efficient as CAT. Oxidized glutathione is reduced back by GPx. In fact, CAT and GPx cooperate in removing \( \text{H}_2\text{O}_2 \) in many tissues\textsuperscript{8,9}.

The normal low rate of production of \( \text{H}_2\text{O}_2 \) by hemoglobin and SOD seems mainly dealt off by GPx. However, CAT also contributes to removal of \( \text{H}_2\text{O}_2 \), if its concentration is raised. All these enzymes convert ROS to less toxic or non-toxic products. The second line of defense to ROS is provided by antioxidant compounds present in the body, such as thiols or taken from outside such as \( \alpha \)-tocopherol, \( \beta \)-carotenoids, flavonoids and L-arginine. We have earlier shown that administration of vitamins C and E to the patients of ischemic myocardial syndromes lowers lipid peroxidation, increases the activities of free radical scavenging enzymes, which are decreased in these patients and lowers the activity of pro-oxidant enzyme which are elevated in the patients\textsuperscript{10-12}.

Oxidized low density lipoproteins are involved in the progression of atherosclerotic lesions which can be prevented by L-arginine administration. Moreover, evidences suggest that L-arginine preserves arterial vasodilation even in the presence of oxidative stress\textsuperscript{14,16}. A fine balance between ROS and various anti-oxidant mechanisms is crucial for avoiding myocardial injury\textsuperscript{17}.

In the present study, we have investigated the effect of oral administration of L-arginine (3 g/day) on the intracellular redox status of the patients of ischemic cardiomyopathy (IC) patients aged 45-60 yrs. The enzymatic and non-enzymatic antioxidant parameters like superoxide dismutase (SOD), catalase (CAT), total thiols and ascorbic acid along with pro-oxidant parameters, such as xanthine oxidase (XO), as well as protein carbonyl content and malondialdehyde (markers of oxidative stress) were investigated in the plasma and RBC lysate.

**Materials and Methods**

**Chemicals**

All the chemicals employed in the study were of Analar grade of Qualigens. Biochemicals were procured from Sigma Chemical Co., USA.

**Subjects**

Patients from Cardiology Department, CSM Medical University and Balarampur Hospital, Lucknow, U.P., India were the candidates enrolled in the study. Informed consent was obtained from each patient. The study was cleared by the Departmental Ethical Committee. The study protocol conformed to the Ethical Guidelines of the 1975 declaration of Helsinki. Patients hospitalized with confirmed diagnosis of myocardial infarction (ST segment elevation/non-ST segment elevation) before reperfusion therapy were included in the ischemic cardiomyopathy (IC) group of the study. These patients had increased levels of cCreatine phosphokinase-MB (CPK-MB) and troponin T. The criteria for exclusion included patients with previous cardiovascular or other organic diseases, such as diabetes, chronic renal failure, left ventricular failure, chronic obstructive pulmonary diseases (COPD), previous history of surgery or trauma and those planned for coronary revascularization.

The subjects were grouped as follows: Group I: non-diabetic, non-smoking 60 healthy persons; Group II: non-diabetic, non-smoking 50 healthy persons from group I administered with oral dose of L-arginine (3 g/day) for 7 days; Group III: non-diabetic, non-smoking 45 healthy persons from group II administered with oral dose of L-arginine (3 g/day) for 15 days; Group IV: 40 ischemic cardiomyopathy (IC) patients assigned to regular therapy that included \( \beta \)-blockers, statins and ACE inhibitors; Group V: 38 patients from group IV receiving regular therapy along with L-arginine administration (3 g/day) for 7 days; and Group VI: 32 patients from group IV receiving regular therapy...
along with L-arginine administration (3 g/day) for 15 days.

The dose selection of L-arginine for the study was as recommended by Fried et al.\textsuperscript{18} and Adams et al.\textsuperscript{19}. 3 g of powdered L-arginine (Sigma Chemical Co., USA) in capsule form was provided to the subjects. Doses above 3 g/day caused mild to moderate headache and nausea in the subjects, thus, the dose was restricted to 3 g, which did not exhibit any of these symptoms, and hence selected as an optimum dose.

Sample preparation and biochemical estimation
The venous blood (4.0 ml) was drawn aseptically from the subjects under study and transferred in polypropylene tubes containing 0.5 ml 3.8% (w/v) sodium citrate, pH 7.2. The tubes were gently rotated to mix the contents and centrifuged at 2000 × g for 20 min at 4°C. The supernatant plasma was used for the biochemical assays.

SOD was assayed by the method described elsewhere\textsuperscript{20}. One unit of the enzyme activity was defined as the amount that caused a 50% inhibition of auto-oxidation of epinephrine in the assay system by 1 ml enzyme preparation. Xanthine oxidase (XO) was assayed by the method described earlier\textsuperscript{21} and one unit of enzyme activity was defined as amount of enzyme that converted 1 µ mole of xanthine to uric acid in 1 min at specified conditions of assay.

Lipid peroxidation, an index of oxidative stress was estimated as malondialdehyde (MDA)\textsuperscript{22}. Plasma ascorbate level was estimated as described previously\textsuperscript{23}. Total thiols were estimated using Ellman’s reagent\textsuperscript{24}. Protein carbonyl content in plasma was also estimated\textsuperscript{25}. Protein estimation was done by the method using Folin phenol reagent\textsuperscript{26}. Specific activity of the enzymes was defined as unit/mg protein.

Statistical analysis
The results were expressed as mean ± SD. One-way analysis of variance (ANOVA), followed by Newman-Keuls multiple comparison test was applied to test the significance of the data and p<0.05 was considered statistically significant.

Results
Ischemia to myocardium is invariably associated with alterations in oxidant and anti-oxidant parameters. In this study, levels of SOD, XO, MDA, protein carbonyl content, total thiols (TSH), ascorbic acid and serum cholesterol were investigated before and after administration of L-arginine. Biochemical parameters investigated are presented in Table 1.

Plasma and RBC lysate parameters

**SOD**
SOD activity in plasma of IC patients (Group IV) decreased by 40% when compared to the healthy subjects (Group I). L-Arginine supplementation to the patients of Group IV for 7 and 15 days increased the activity by 10.4% and 40%, respectively (Group IV vs V; p = ns, + 10.4% and Group IV vs Group; p<0.001, + 40%). The healthy subjects of group I also showed elevated activity upon L-arginine supplementation (Table 1).

SOD activity in RBC lysate of IC patients was reduced by 38% when compared to the healthy subjects (Group I vs IV; p<0.001, -38%). L-Arginine supplementation to the patients of group IV for 7 and 15 days increased the activity by 20% and 37%, respectively (Group IV vs V; p = ns, + 20% and Group IV vs VI; p<0.001, + 37%). The healthy subjects of group I also showed elevated activity upon L-arginine supplementation (Group I vs II; p<0.001, + 19% and Group I vs III; p<0.001, + 34%) (Table 1).

**TSH**
As shown in Table 1, total thiol level was reduced significantly in the IC patients when compared to the healthy subjects (Group I vs IV; p<0.001, -66%). L-Arginine therapy to the patients for 7 and 15 days increased the TSH level significantly (Group IV vs V; p<0.05, + 28% and Group IV vs VI; p<0.001, + 66.7%). L-Arginine supplementation also improved the TSH level in the healthy subjects (Group I vs II; p<0.05, + 17% and Group I vs III; p<0.001, + 47%).

TSH level in RBC lysate of IC patients was also reduced significantly when compared to the healthy subjects (Group I vs IV; p<0.001, -76.6%). L-Arginine therapy to the patients for 7 and 15 days increased the TSH level significantly (Group IV vs V; p<0.001, + 70.32% and Group IV vs VI; p<0.01, + 61%). In healthy subjects also, the TSH level improved upon L-arginine supplementation (Group I vs II; p = ns, + 11% and Group I vs III; p<0.01, + 28%).

**Ascorbic acid**
Plasma ascorbate level in IC patients was reduced by 55% when compared to the healthy subjects (Group I vs
IV; p<0.001, -55%). L-Arginine supplementation to the patients for 7 days improved the ascorbate level by 28% (Group IV vs V; p = ns) and by 48% upon 15 day of the therapy (Group IV vs VI; p<0.001). Ascorbate level also increased in the healthy subjects, but not significantly (Table 1).

**XO**

As shown in Table 1, XO (a pro-oxidant enzyme) activity increased by 37% in IC patients, as compared to the healthy subjects (Group I vs IV; p<0.001). L-Arginine therapy for 7 and 15 days reduced the XO activity in the patients by 14% and 25%, respectively (Group IV vs V; p = ns and Group IV vs VI; p<0.001). In the healthy subjects also, XO activity reduced upon L-arginine therapy (Group I vs II; p<0.01, -30 % and Group I vs III; p<0.01, -39%).

**Table 1—Parameters in plasma and RBC lysate of subjects at 7th and 15th day of L-arginine administration**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0 day (n = 60)</th>
<th>7 day (n = 50)</th>
<th>15 day (n = 45)</th>
<th>0 day (n = 40)</th>
<th>7 day (n = 38)</th>
<th>15 day (n = 32)</th>
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<tbody>
<tr>
<td><strong>Plasma</strong></td>
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<td>Antioxidants</td>
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<tr>
<td>SOD (U/mg protein)</td>
<td>2.98 ± 0.13</td>
<td>3.74 ± 0.23</td>
<td>4.28 ± 0.16</td>
<td>1.83 ± 0.09</td>
<td>2.02 ± 0.10</td>
<td>2.56 ± 0.10</td>
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<tr>
<td>TSH (nmole/ml)</td>
<td>0.53 ± 0.03</td>
<td>0.62 ± 0.04</td>
<td>0.78 ± 0.08</td>
<td>0.18 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.30 ± 0.02</td>
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<tr>
<td>Ascorbic acid (mg/dl)</td>
<td>0.65 ± 0.05</td>
<td>0.71 ± 0.07</td>
<td>0.72 ± 0.07</td>
<td>0.29 ± 0.02</td>
<td>0.37 ± 0.02</td>
<td>0.43 ± 0.03</td>
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<td><strong>Pro-oxidants</strong></td>
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<td>XO (U/mg protein)</td>
<td>0.57 ± 0.03</td>
<td>0.40 ± 0.04</td>
<td>0.34 ± 0.02</td>
<td>1.01 ± 0.04</td>
<td>0.90 ± 0.03</td>
<td>0.68 ± 0.03</td>
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<td><strong>Oxidants</strong></td>
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<td>MDA (nmole/ml)</td>
<td>1.46 ± 0.08</td>
<td>1.27 ± 0.06</td>
<td>1.12 ± 0.07</td>
<td>4.84 ± 0.22</td>
<td>4.22 ± 0.27</td>
<td>3.73 ± 0.27</td>
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<tr>
<td>Protein carbonyl (µmole/ml)</td>
<td>21.32 ± 1.82</td>
<td>20.09 ± 2.07</td>
<td>19.26 ± 2.02</td>
<td>53.20 ± 4.38</td>
<td>44.70 ± 4.43</td>
<td>37.20 ± 3.29</td>
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<tr>
<td><strong>RBC lysate</strong></td>
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<tr>
<td>Antioxidants</td>
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<tr>
<td>SOD</td>
<td>6.42 ± 0.19</td>
<td>7.64 ± 0.264</td>
<td>8.59 ± 0.26</td>
<td>3.98 ± 0.19</td>
<td>4.77 ± 0.16</td>
<td>5.47 ± 0.13</td>
</tr>
<tr>
<td>TSH</td>
<td>4.75 ± 0.26</td>
<td>5.26 ± 0.43</td>
<td>6.07 ± 0.46</td>
<td>1.23 ± 0.99</td>
<td>1.66 ± 0.11</td>
<td>1.93 ± 0.12</td>
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<tr>
<td><strong>Pro-oxidants</strong></td>
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<tr>
<td>XO</td>
<td>2.35 ± 0.22</td>
<td>1.95 ± 0.16</td>
<td>1.53 ± 0.13</td>
<td>3.47 ± 0.15</td>
<td>3.01 ± 0.14</td>
<td>2.61 ± 0.12</td>
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<tr>
<td><strong>Oxidants</strong></td>
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<tr>
<td>MDA</td>
<td>48.01 ± 2.45</td>
<td>39.47 ± 3.07</td>
<td>34.51 ± 2.53</td>
<td>93.46 ± 2.83</td>
<td>90.39 ± 4.61</td>
<td>82.18 ± 2.79</td>
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**p-values:** p<0.05; significant

**SOD:**
In plasma, Group I vs II, p<0.001, Group I vs III, p<0.001, Group I vs IV, p<0.001, Group IV vs V, p>0.05, Group IV vs VI, p<0.001. In RBC lysate, Group I vs II, p<0.001, Group I vs III, p<0.001, Group I vs IV, p<0.001, Group IV vs V, p<0.01, Group IV vs VI, p<0.001.

**TSH:**
In plasma, Group I vs II, p<0.05, Group I vs III, p<0.001, Group I vs IV, p<0.001, Group IV vs V, p<0.05, Group IV vs VI, p<0.001. In RBC lysate, Group I vs II, p>0.05, Group I vs III, p<0.05, Group I vs IV, p<0.001, Group IV vs V, p<0.05, Group IV vs VI, p<0.01.

**Ascorbic acid:**
In plasma, Group I vs II, p>0.05, Group I vs III, p<0.05, Group I vs IV, p<0.001, Group IV vs V, p>0.05, Group IV vs VI, p<0.001.

**XO:**
In plasma, Group I vs II, p<0.001, Group I vs III, p<0.001, Group I vs IV, p<0.001, Group IV vs V, p<0.05, Group IV vs VI, p<0.001. In RBC lysate, Group I vs II, p>0.05, Group I vs III, p<0.01, Group I vs IV, p<0.01, Group IV vs V, p>0.05, Group IV vs VI, p<0.01.

**MDA:**
In plasma, Group I vs II, p>0.05, Group I vs III, p<0.001, Group I vs IV, p<0.001, Group IV vs V, p>0.05, Group IV vs VI, p<0.001. In RBC lysate, Group I vs II, p>0.05, Group I vs III, p<0.01, Group I vs IV, p<0.001, Group IV vs V, p>0.05, Group IV vs VI, p<0.05.

**Protein carbonyl:**
In plasma, Group I vs II, p>0.05, Group I vs III, p<0.05, Group I vs IV, p<0.001, Group IV vs V, p<0.01, Group IV vs VI, p<0.001.
for 7 and 15 days reduced the XO activity in the patients by 13.26% and 25%, respectively (Group IV vs V; p = ns and Group IV vs VI; p < 0.001). In the healthy subjects also, XO activity reduced upon L-arginine therapy (Group I vs II; p = ns, -17% and Group I vs III; p < 0.01, -35%) (Table 1).

MDA

The IC patients exhibited 231% elevated MDA level, as compared to the healthy subjects (Group I vs IV; p < 0.001). L-Arginine treatment for 7 and 15 days to these patients reduced their MDA level (Group IV vs V; p = ns, -13% and Group IV vs VI; p < 0.001, -25%). In the healthy subjects also, MDA level reduced upon L-arginine supplementation (Group I vs II; p = ns, -13% and Group I vs III; p < 0.001, -23.3%) (Table 1).

In the RBC lysate of IC patients, MDA level exhibited 95% elevation as compared to the healthy subjects (Group I vs IV; p < 0.001). L-Arginine treatment for 7 and 15 days to these patients reduced their MDA level (Group IV vs V; p = ns, -4% and Group IV vs VI; p < 0.05, -19%). In the healthy subjects also, MDA level reduced upon L-arginine supplementation (Group I vs II; p = ns, -18% and Group I vs III; p < 0.01, -28%) (Table 1).

Protein carbonyl content

Carbonyl content in the plasma of IC patients increased by 149% when compared to the healthy subjects (Group I vs IV; p < 0.001). L-Arginine therapy for 7 and 15 days reduced the carbonyl content of these patients (Group IV vs V; p < 0.01, -16% and Group IV vs VI; p < 0.001, -31%). Healthy subjects exhibited slight reduction in their plasma carbonyl content (Table 1).

Discussion

In our study, most of the oxidant-antioxidant parameters showed a tendency to improve upon L-arginine administration. Most of the xanthine oxidizing activity present in healthy animal tissues occurs as a dehydrogenase enzyme that oxidizes hypoxanthine and/or xanthine to uric acid at the expense of NAD. During ischemia, insufficient oxygen supply fails to support oxidative phosphorylation, leading to lowering of tissue ATP levels and acidosis. ATP hydrolysis eventually leads to production of hypoxanthine and xanthine. Thus, there is accumulation of substrates of XO. The levels of Ca are also increased during ischemia which stimulates certain proteases that selectively act on xanthine dehydrogenase to convert it in to XO.

XO-mediated production of oxygen free radicals plays major role in causing tissue damage observed in ischemia/reperfusion injury, as evidenced by the fact that incorporation of specific inhibitors of XO (such as allopurinol and anti-oxidants) are capable to protect such damage in experimental models of myocardial injury. The series of events initiated by ischemia lead to enhanced production of free radicals that further increase upon reperfusion of ischemic myocardium. Oxy free radicals are removed by SOD in healthy organisms, but during ischemia, the lowered activity of SOD caused by inhibition of the enzyme by excess H₂O₂ production fails to cope with excessive production of free radicals and H₂O₂. This excess H₂O₂, besides inhibiting SOD, can cause degradation of heme rings of hemoglobin releasing iron which is capable of OH⁻ production via Fenton reaction and lipid peroxidation.

In the present study, lipid peroxidation and free radical-mediated damage during ischemia was indicated by increased MDA levels in patients suffering from myocardial infarction and acute angina. Total thiols can either chemically or enzymatically reduce dehydroascorbic acid (DHA) to ascorbate. Ascorbic acid, an effective antioxidant is capable of the decreased conversion of DHA back to ascorbate. Decrease in ascorbate level fails to cope with oxidative damage of lipids and modification of phospholipids, thereby resulting in increased lipid peroxidation. Decreased levels of total thiols might be responsible for the decreased conversion of DHA back to ascorbate. Ascorbic acid, an effective antioxidant is capable of completely protecting lipids against oxidative damage induced by peroxo radicals. It has chain breaking properties, reacts directly with superoxide ions, hydroxyl radicals and singlet oxygen species and acts to generate tocopherol. During ischemia, the lowered levels of total thiols might be responsible for decreased conversion of DHA to ascorbate. This might be one of the reasons that decreased levels of ascorbic acid were observed in the patients in the present study.

Decrease in ascorbate level fails to cope with peroxidative damage of lipids and modification of phospholipids, thereby resulting in increased lipid peroxidation. Decreased levels of total thiols also indicated the effect of oxidative damage of protein molecules, due to excessive free radical generation during ischemia. Major parts of thiols are derived from proteins. Oxidative damage modifies proteins, leading to oxidation of thiol groups. In present study, modifications in lipids and proteins caused due to decreased levels of ascorbic acid and thiols in patients altered membrane permeability and configuration, in addition to producing functional modification of various cellular proteins during ischemia.
free radical generation during ischemia causes the inactivation of enzymes and introduction of carbonyl groups into amino acid side chains of proteins. Increased carbonyl derivatives within proteins and enzyme inactivation suggest mixed-function oxidative modification.

During ischemia, myocardial tissue is exposed to an exacerbated injury caused by un-scavenged superoxide radicals, other free radicals, increased WBC, vasoconstriction and platelet adhesion. Higher oxidant stress and diminished antioxidant status along with higher MDA levels constitute the key factors in the progression of ischemic injury. L-Arginine serves as a precursor of nitric oxide (NO) synthesis. NO can reduce oxidative stress by inhibiting XO, scavenge superoxide radicals and terminate free radical chain reaction within the lipid membranes, thereby reducing inflammatory mediators. Decreased ROS formation attenuates inhibition of SOD which is increased by L-arginine supplementation. In the present study, L-arginine supplementation also increased the ascorbic acid and total thiols levels, which cope with lipid and protein peroxidation, thus decreasing the damage of membrane and cellular proteins. By efficiently reducing or eliminating these toxic metabolites, L-arginine via NO retards the damaging effects of ischemic injury. In this study, L-arginine controlled the modification of proteins due to excess carbonylation, resulting in the significantly decreased carbonyl levels in the plasma of patients.

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