Therapeutic role of L-arginine on free radical scavenging system in ischemic heart diseases

Pratima Tripathi and M K Misra*
Department of Biochemistry, Lucknow University, Lucknow 226007, India

Received 31 July 2009; revised 24 November 2009

Increased production of free radicals under oxidative stress conditions plays a vital role in the impairment of endothelial function and also in the pathogenesis of ischemic heart diseases. Ischemia, followed by reperfusion, leads to the exacerbated formation of oxy-free radicals. These reactive oxygen species through a chain of reactions damage the cardiomyocytes and cause more injury to the myocardium. L-Arginine is reported to act as free radical scavenger, inhibits the activity of pro-oxidant enzymes and thus acts as an antioxidant and these roles of L-arginine are mediated by nitric oxide (NO). In the present study, the effect of oral administration of L-arginine (3 g/day for 7 days) on some antioxidant enzymes, total thiols, lipid peroxidation measured as malondialdehyde (MDA), and plasma ascorbate levels in myocardial ischemic patients was investigated. We observed an increase in the activity of superoxide dismutase (SOD), total thiols (T-SH) and plasma ascorbate levels and a decrease in the activity of xanthine oxidase (XO), MDA levels, carbonyl content and serum cholesterol in the patients on oral administration of L-arginine. The present study demonstrates that L-arginine administration may be beneficial to patients with myocardial ischemic disorders, such as acute myocardial infarction and acute angina.

Keywords: Acute myocardial infarction, Acute angina, Ischemia, Oxidative stress, L-Arginine, Nitric oxide, Superoxide dismutase, Xanthine oxidase, Malondialdehyde, Ascorbic acid.

Myocardial ischemia occurs generally due to infarct formation in the vessels supplying blood to the myocardium. Infarction reduces the supply of oxygen required for proper functioning of the heart and thus resulting in myocardial damage. Shortage of oxygen (hypoxia) produces depression in myocardial contractile function and alterations in Ca\(^{2+}\) homeostasis. Upon reperfusion, sudden burst of oxygen to the myocardium leads to oxidative stress with consequent enhanced formation of reactive oxygen species (ROS). Excessive formation of ROS results in the oxidation of proteins and lipids causing damage to the contractile proteins of the sarcolemma, thereby reducing the Ca\(^{2+}\) sensitivity of the sarcolemma, leading to failure of Ca\(^{2+}\) regulatory mechanisms and ultimately intracellular Ca\(^{2+}\) and Na\(^+\) overload and cell death. During ischemia, an increase in Ca\(^{2+}\) induces conversion of xanthine dehydrogenase to xanthine oxidase and subsequent generation of superoxide radicals. The superoxide radicals trigger the formation of other highly reactive oxygen species like OH, H\(_2\)O\(_2\) and HOCl which damage and result in the loss of membrane integrity.

To counter the damaging effects of ROS, the body has evolved certain defense mechanisms like enzymatic free radical scavengers such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) that convert the toxic oxygen metabolites to non-toxic products. The effects of the oxy-free radicals could also be suppressed by supplementation of some antioxidant compounds like α-tocopherol, β-carotenoids, flavonoids and of late L-arginine. A fine balance between the antioxidant defense system of the body and ROS is essential for avoiding myocardial damage. L-Arginine is the precursor of nitric oxide (NO) which has been identified as the endothelial derived relaxing factor. The NO causes substantial vasorelaxation, inhibits platelet adhesion and aggregation, reduces neutrophil interaction with the endothelium and may neutralize superoxide radicals.

L-Arginine is shown to reduce infarct size in the ischemic regions and preserve endothelial function and also has been shown to have antioxidant...
properties. In the present study, the effect of L-arginine administration has been evaluated on some oxidant-antioxidant parameters in myocardial ischemia, represented by acute myocardial infarction (AMI) and acute angina (AA). Such a study, in the present context has not been reported earlier.

Materials and Methods

Chemicals
All the chemicals employed were AnalaR grade of Qualigens and biochemicals were procured from Sigma Chemical Co., USA.

Subjects and treatment
Subjects and treatment was approved by the Departmental Ethical Committee and the informed consent was obtained from individuals enrolled in the study. The study included 60 healthy persons, 35 patients suffering from acute myocardial infarction and 25 patients from acute angina. The subjects included in the study were in the age group of 40-75 yrs. Patients with previous cardiovascular or other organic diseases such as diabetes and those with severe trauma or surgery in preceding 8 weeks were excluded from the study. The AMI group of the study included the patients suffering from non-segment elevation myocardial infarction before reperfusion. These patients were receiving anti-hypertensive drugs viz. β-blockers, ACE inhibitors and aspirin.

The AA group included the patients with chest pain at rest and/or on effort, positive exercise tests (chest pain associated with ST-segment depression of >0.1 mV), normal coronary arteriograms and no spasm of large epicardial arteries provoked by acetylcholine. All these patients exhibited evidence, suggestive of myocardial ischemia (myocardial lactate production, chest pain or ischemic ST-segment abnormalities). None of these patients had any ST-segment abnormalities in their resting ECG’s. All these patients were receiving regular antihypertensive medicines viz. nitroglycerine, aspirin, clopivas and monotrate. L-Arginine was supplemented orally in a dose of 3 g per day for 7 days to all the cases as the standard protocol.

The subjects were grouped as follows: Group I: non-diabetic, non-smoking 60 healthy persons; Group II: non-diabetic, non-smoking 45 healthy persons administered with oral dose of L-arginine (3 g/day) for 7 days; Group III: non-diabetic, non-smoking 35 AMI patients receiving regular therapy without L-arginine administration; Group IV: non-diabetic, non-smoking 30 AMI patients assigned to regular therapy along with supplementation of oral dose of L-arginine (3 g/day) for 7 days; Group V: non-diabetic, non-smoking 25 AA patients receiving regular therapy without L-arginine administration; and Group VI: non-diabetic, non-smoking 20 AA patients receiving regular therapy along with L-arginine (3 g/day) administration for 7 days.

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. One unit of enzyme activity was defined to cause 50% inhibition of autooxidation of epinephrine in the assay system by 1 ml enzyme preparation. XO was assayed by the method described earlier. 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 in the form of malondialdehyde (MDA) formed. Plasma ascorbate level was estimated by the method of Omaye et al. Total thiols were estimated using Ellman’s reagent. Protein carbonyl content in plasma and serum cholesterol level was also estimated. Protein estimation was done by the method using Folin phenol reagent. Specific activity of 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. A p<0.05 was considered statistically significant.

Results
Data for age and blood pressure of the subjects included in the study are provided in Table 1. From these data, it is clear that the blood pressure of AMI and AA patients was found to be greater than the control subjects.

Table 2 shows that the specific activity of SOD in patients with AMI as well as AA receiving L-arginine treatment along with regular antihypertensive therapy was found to increase, though insignificantly when compared to those receiving only antihypertensive
therapy (p>0.05). Similarly, the control subjects receiving L-arginine also showed an insignificant increase in the specific activity of SOD when compared to those receiving no therapy (p>0.05). The specific activity of XO and levels of MDA in the patients receiving L-arginine treatment along with regular antihypertensive therapy was found to decrease when compared to those receiving only anti-hypertensive therapy and the decrease was statistically significant in case of XO (p<0.05 for AMI vs AMI + Arg), but non-significant (p>0.05) for AA vs AA + Arg and in case of lipid peroxidation. The control subjects receiving L-arginine also showed significant reduction in the specific activity of XO (p<0.005) and non-significant reduction of LPO (p>0.05) when compared to those receiving no L-arginine.

The levels of total thiols and ascorbic acid in plasma of patients with AMI as well as AA receiving L-arginine treatment along with regular antihypertensive therapy are presented in Table 3. These parameters were found to increase insignificantly (p>0.05 for both the cases), when compared to those receiving only anti-hypertensive therapy. The control subjects receiving L-arginine also showed elevated levels of total thiols and ascorbate in their plasma, when compared to those receiving no therapy and the increase was statistically significant (p<0.05) in case of total thiols but not significant in case of ascorbate (p>0.05).

As shown in Table 3, carbonyl content and serum cholesterol in plasma of patients with AMI as well as AA receiving L-arginine treatment along with regular anti-hypertensive therapy are presented in Table 3. These parameters were found to increase insignificantly (p>0.05 for both the cases), when compared to those receiving only anti-hypertensive therapy. The control subjects receiving L-arginine also showed elevated levels of total thiols and ascorbate in their plasma, when compared to those receiving no therapy and the increase was statistically significant (p<0.05) in case of total thiols but not significant in case of ascorbate (p>0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
<th>XO (U/mg protein)</th>
<th>MDA (n mole/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.99 ± 1.42</td>
<td>0.57 ± 0.23</td>
<td>1.46 ± 0.56</td>
</tr>
<tr>
<td>II</td>
<td>3.24 ± 1.55</td>
<td>0.40 ± 0.23</td>
<td>1.27 ± 0.33</td>
</tr>
<tr>
<td>III</td>
<td>1.91 ± 0.79</td>
<td>0.94 ± 0.17</td>
<td>4.61 ± 1.27</td>
</tr>
<tr>
<td>IV</td>
<td>2.08 ± 0.63</td>
<td>0.82 ± 0.21</td>
<td>4.20 ± 1.23</td>
</tr>
<tr>
<td>V</td>
<td>2.80 ± 0.71</td>
<td>0.81 ± 0.15</td>
<td>1.91 ± 0.75</td>
</tr>
<tr>
<td>VI</td>
<td>2.91 ± 0.69</td>
<td>0.74 ± 0.13</td>
<td>1.67 ± 0.58</td>
</tr>
</tbody>
</table>

Statistical significance of the groups was determined by one-way ANOVA, followed by Newmann-Keuls multiple comparison test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total thiols (n mole/ml)</th>
<th>Ascorbic acid (mg/dl)</th>
<th>Carbonyl content (µ mole/ml)</th>
<th>Serum cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.30 ± 0.14</td>
<td>0.65 ± 0.32</td>
<td>21.32 ± 10.11</td>
<td>163.60 ± 39.38</td>
</tr>
<tr>
<td>II</td>
<td>0.39 ± 0.18</td>
<td>0.68 ± 0.26</td>
<td>20.09 ± 10.95</td>
<td>148.40 ± 36.43</td>
</tr>
<tr>
<td>III</td>
<td>0.22 ± 0.07</td>
<td>0.29 ± 0.08</td>
<td>54.28 ± 22.92</td>
<td>411.90 ± 74.81</td>
</tr>
<tr>
<td>IV</td>
<td>0.24 ± 0.06</td>
<td>0.30 ± 0.12</td>
<td>47.10 ± 22.22</td>
<td>340.00 ± 54.73</td>
</tr>
<tr>
<td>V</td>
<td>0.32 ± 0.04</td>
<td>0.40 ± 0.09</td>
<td>21.47 ± 11.57</td>
<td>335.90 ± 64.25</td>
</tr>
<tr>
<td>VI</td>
<td>0.36 ± 0.06</td>
<td>0.43 ± 0.11</td>
<td>20.36 ± 11.79</td>
<td>304.40 ± 59.70</td>
</tr>
</tbody>
</table>

Statistical significance of the groups was determined by one way ANOVA followed by Newmann-Keuls multiple comparison test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (Yrs)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>50 ± 10</td>
<td>118 ± 3/79 ± 4</td>
</tr>
<tr>
<td>II</td>
<td>50 ± 10</td>
<td>120 ± 2/80 ± 2</td>
</tr>
<tr>
<td>III</td>
<td>60 ± 10</td>
<td>138 ± 10/77 ± 4</td>
</tr>
<tr>
<td>IV</td>
<td>60 ± 10</td>
<td>115 ± 5/80 ± 9</td>
</tr>
<tr>
<td>V</td>
<td>55 ± 10</td>
<td>126 ± 14/81 ± 10</td>
</tr>
<tr>
<td>VI</td>
<td>55 ± 10</td>
<td>116 ± 5/77 ± 5</td>
</tr>
</tbody>
</table>

Table 2—Effect of L-arginine (Arg) administration on the activities of SOD, XO and MDA levels in control subjects and in the patients of AMI and AA

[Values represent mean ± SD]
AA receiving L-arginine treatment along with regular antihypertensive therapy was found to decrease, when compared to those receiving only anti-hypertensive therapy, although the decrease in case of carbonyl content was statistically not significant (p>0.05) while for serum cholesterol the decrease was statistically significant (p<0.005 for AMI + Arg vs AMI), and non-significant (p>0.05) for AA + Arg vs AA. The control subjects receiving L-arginine therapy also showed reduced carbonyl content and serum cholesterol in their plasma, when compared to those receiving no therapy, but the decrease was statistically not significant (p>0.05).

Discussion
In the present study, most of the oxidant-antioxidant parameters improved following L-arginine administration. Inadequate oxygen supply to the myocardium during ischemia leads to the conversion of xanthine dehydrogenase to XO, which upon reperfusion catalyzes conversion of hypoxanthine and xanthine to uric acid with excessive production of oxy-free radicals. These are converted by SOD to more toxic H$_2$O$_2$, which in turn is converted to H$_2$O by catalase and/or GPx. In healthy organisms, the endogenous enzymatic antioxidant system comprising of SOD, CAT, GPx and GR is responsible for metabolic homeostasis and controls the free radical overproduction, but under ischemic conditions, this antioxidant system is eroded and the conversion of H$_2$O$_2$ into destructive OH is increased via Fenton reaction.$^{22}$

It is well-known that thiols can both chemically and enzymatically reduce dehydroascorbic acid (DHA) back to ascorbate.$^{23,24}$ Also, ascorbic acid is shown to have protective effect against the peroxidative damage of lipids.$^{25}$ Increased oxidative stress conditions, as evident from higher MDA levels lead to increased consumption of ascorbic acid.$^{26,27}$ Low levels of thiols and increased consumption of ascorbic acid during oxidative stress might be the reason for low ascorbic acid level in patients with myocardial ischemic syndromes. Excessive free radical generation during ischemia causes inactivation of the enzymes and introduction of carbonyl groups into the amino acid side chains of proteins.$^{28}$ Excess carbonyl derivatives within proteins suggest increased protein oxidation.$^{29}$ The patients included in the present study were also found to have higher serum cholesterol levels which significantly decreased after L-arginine administration.

During ischemia, the myocardial tissue is exposed to damage caused by unscavenged superoxide radicals, other free radicals, increased WBC, vasoconstriction and excessive platelet adhesion. All these factors together accelerate the oxidative injury, followed by diminished antioxidant status, increased lipid and protein oxidation and hence progression of the ischemic injury. L-Arginine has an indirect antioxidant role and is a naturally occurring continuously released vasoactive agent that controls coronary vascular tone.$^{29}$ NO is synthesized by the vascular endothelium through the conversion of L-arginine to L-citrulline by NO synthase.$^{30}$ It has been found to be cardioprotective in ischemia-reperfusion$^{31,32}$ and exerts direct effect on cardiac myocytes.$^{33}$ L-Arginine through NO has an indirect antioxidant role to scavenge oxy-free radicals and inhibit XO activity, thus reducing ROS formation.$^{34-36}$

In our study, the activity of XO was found to be reduced after L-arginine supplementation. Reduction in XO activity leads to decreased formation of O$_2^\cdot$ and H$_2$O$_2$. L-Arginine indirectly triggers the activity of antioxidant enzyme SOD by controlling the excessive formation of H$_2$O$_2$, which otherwise inhibits the enzyme when in abundance during stress conditions. This might be the reason for increased activity of SOD in L-arginine supplemented groups of our study.

Thiols, the major non-enzymatic antioxidant play an important role in protecting the myocardium from free radicals-induced oxidative damage.$^{37}$ Inhibition of free radical generation by L-arginine prevents the oxidation of thiols found in the membranes and hence the levels of total thiols were found to increase after the supplementation of L-arginine. Increase in thiol levels promotes the conversion of DHA to ascorbate and also reduces the oxidative stress induced lipid and protein peroxidation.$^{35}$ After L-arginine supplementation, the levels of ascorbic acid increased in the subjects under study, while the levels of MDA and carbonyl content were found to be reduced. NO formed through L-arginine also reduces the serum cholesterol levels$^{38-41}$. NO also inhibits vasoconstriction, expression of adhesion molecules and chemotactic proteins$^{42,43}$, thereby controlling the blood pressure and infarction in patients of myocardial ischemic syndromes.

To conclude, the present study demonstrates the beneficial effects of L-arginine administration in myocardial ischemic conditions. L-Arginine supplementation, therefore, may be useful in the development of therapeutic strategies, aiming to reduce
oxidative stress conditions caused during myocardial ischemic syndrome.

Acknowledgements
One of us (P T) is thankful to CST, U.P. for a Fellowship. Thanks are due to DST for financial assistance to the department under FIST program.

References
15 Fried R & Fried L W (1945) Meth Enz Anal 2, 644-649
16 Ohkawa H, Oshishi N & Yagi K (1979) Anal Biochem 95, 351-358
17 Omaye S T, Turnbull J D & Sauberlich H E (1979) Meth Enzymol 62, 3-11
23 Basu S, Som S, Mukherjee D & Chatterjee I B (1979) Biochim Biophys Acta 1090, 1335-1340