Protective Role of L-Arginine Against Free-Radical Mediated Oxidative Damage in Patients with Unstable Angina

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Abstract Unstable angina is a critical condition of heart resulting from narrowing of vessels supplying blood to heart. Ischemia of the myocardium leads to oxidative stress and severe tissue damage. The objective of the present study was to determine the effect of L-arginine administration on the oxidant–antioxidant homeostasis which otherwise gets imbalanced in patients with cardiovascular diseases. The results obtained, show improvement in the oxidant–antioxidant levels of the subjects upon incorporation of L-arginine. Our findings suggest that supplementation of L-arginine along with regular anti-anginal therapy may be beneficial to the patients of unstable angina.

Keywords Unstable angina · Oxidative stress · L-arginine · Oxidant–antioxidant homeostasis · Reactive oxygen species

Introduction

L-arginine, the physiological substrate of nitric oxide (NO) synthesis, improves endothelium dependent vasodilatation in hyper-cholesterolemic humans [1, 2] and in animal models has anti-atherogenic action and reduces oxidative stress [3], platelet aggregation [4, 5], monocyte adhesion [6] and formation of initial lesions [7]. Decreased platelet aggregation has also been observed in humans [8]. When

given orally, L-arginine improves symptoms of claudication in patients with peripheral vascular disease [9]. Beneficial effects of L-arginine are through increased NO production [10]. In recent years it has become apparent that dynamic aspects of vascular physiology, mainly endothelium, plays a major role both in the pathogenesis and clinical manifestations of coronary artery disease [11]. Endothelial dysfunction results in reduced availability of NO, which results not only in atherogenesis but also to rapid coronary artery disease progression and acute coronary events [12]. Experimental and clinical studies have shown that NO plays an important anti-atherogenic role and in its absence, the genesis and progression of atherosclerosis are facilitated [13]. The loss of protective action of NO leads to increased release of vasoconstrictor substances, monocyte recruitment into the arterial intima, expression of surface adhesion molecules and production of growth factors that promote vascular smooth muscle cell proliferation and migration. Enhanced thrombogenicity and decreased fibrinolysis also results from endothelial dysfunction [14, 15]. In patients with unstable angina endothelial dysfunction is apparent and might result in atherogenesis and hence narrowing of blood vessels. All these contribute to the pathogenesis of the condition and the clinical instability of the patients.

The purpose of the present study was to determine whether L-arginine has any effect on pro-oxidant and anti-oxidant homeostasis in the patients with unstable angina.

Materials and Methods

The study was cleared by the departmental ethical committee. Informed consent was obtained from all the individuals enrolled in the study. The patient group of unstable

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angina exhibited chest pain at rest/or on effort and positive exercise tests. These patients were receiving anti-anginal medications like aspirin, β -blockers and angiotensin converting enzyme (ACE) inhibitors. Patients with previous cardiovascular or other organic diseases and those with severe trauma or surgery in preceding 8 weeks were excluded from the study. The subjects were grouped as follows:

- Group 1: included 55 healthy persons with no L-arginine therapy serving as control.
- Group 2: included forty healthy persons with oral dose of L-arginine (3 g/day) for 15 days.
- Group 3: had twenty five individuals suffering from unstable angina and receiving anti-anginal medication.
- Group 4: comprised of twenty individuals with unstable angina, receiving oral L-arginine (3 g/day) for 15 days along with routine anti-anginal therapy.

All the chemicals employed in the study were AnalaR grade of Qualigens. Biochemicals were procured from Sigma Chemical Co., USA. The dose selection of L-arginine for the study was as recommended by Fried et al. [14] and Adams et al. [15]. Doses above 3 g/day caused mild to moderate headache and nausea in the persons, therefore, the dose was restricted to 3 g. which did not exhibit any of these symptoms.

Venous blood (4.0 ml) was drawn aseptically from the subjects and transferred in propylene tubes containing 0.5 ml 3.8% (w/v) sodium citrate pH 7.2. The mixture was centrifuged at $2,000 \times g$ for 20 min at 4°C and the supernatant plasma, thus obtained, was stored for study.

Parameters studied: *Superoxide dismutase (SOD) in plasma* was assayed by the method of Misra and Fridovich [16]. One unit of enzyme activity was defined to cause 50% inhibition of auto-oxidation of epinephrine present in the assay system by 1 ml enzyme preparation.

Xanthine-oxidase (XO) was assayed by the method of Fried and Fried [17]. One unit of enzyme activity was defined as amount of enzyme that converts 1 μ mol of xanthine to uric acid in 1 min under conditions of assay.

Ascorbic acid levels in plasma was estimated by the method of Omaye et al. [18] using L-ascorbic acid in 5% Trichloroacetic acid (TCA) as reference.

Total thiols (T-SH) were estimated by the method of Hu [19] using Ellman's reagent, measuring absorbance at 412 nm.

Lipid peroxidation (LPO) in the form of malondialdehyde (MDA) levels were measured as the index of oxidative stress by the method of Ohkawa et al. [20] using 1,1,3,3 tetra ethoxy propane (TEP) as reference.

Protein-oxidation was measured as carbonyl contents of plasma proteins by the method of Levini et al. [21].

Carbonyl content was calculated using molar extinction coefficient of $22,000 \text{ M}^{-1} \text{ cm}^{-1}$.

Serum cholesterol and triglycerides were determined in the hospital's pathology using enzymatic diagnostic kits, based on the cholesterol-oxidase phenol per-oxidase and glycerol 3-phosphatase peroxide methods. Baseline characteristics of the subjects were obtained from the hospital records.

Protein estimation was done by the method of Lowry et al. [22], using Folin phenol reagent. Specific activity of the enzymes was expressed as unit/mg protein.

Statistical Analysis

The results were expressed as mean $\pm$ SEM. One way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test was applied to assess the significance of the data. $P < 0.05$ is considered to be statistically significant.

Results

As shown in the Tables 1, 2 and 3, almost all the anti-oxidant and pro-oxidant parameters show a significant deviations from the normal values observed in healthy subjects before L-arginine administration. The levels of significance of the data are expressed in the respective tables. The basic characteristics of the control and patient groups like age, sex and blood pressure along with their serum lipid profile have been presented in Table 1. When healthy persons were given L-arginine, there was significant lowering of serum cholesterol levels. Other parameters, like serum triglycerides and LDL also improved but the change was statistically not significant. L-arginine administration to the unstable angina patients did not result in any significant alterations in these parameters. Unstable angina results in significant decrease in anti-oxidant parameters such as SOD activity, total thiols and ascorbic acid (Table 2) and increase in pro-oxidant parameters such as xanthine oxidase activity and MDA levels. These observations emphasize the fact that unstable angina, like other coronary artery diseases, is associated with oxidative stress. A very interesting observation of the study is that the L-arginine administration to patients though does not significantly affects anti-oxidant parameters, is very effective in reducing pro-oxidant parameters (Table 3).

Discussion

Unstable angina is a discomfort condition of heart and occurs when the blood vessels supplying the heart become

Table 1 Baseline characteristics of the subjects before and after L-arginine treatment

	Gr. 1	Gr. 2	Gr. 3	Gr. 4
Number	55	40	25	20
Sex	Male	Male	Male	Male
Age (years)	50 ± 2.54	50 ± 2.54	55 ± 1.65	55 ± 1.65
Diastolic BP (mmHg)	79 ± 0.53	80 ± 0.34	90.25 ± 2.34	81.88 ± 0.64
Systolic BP (mmHg)	120 ± 0.42	119 ± 0.54	128.4 ± 2.53	124 ± 1.94
S. cholesterol (mg/dl)	200.60 ± 7.12	195.10 ± 6.08 ^a	279.70 ± 8.83 ^b	264.80 ± 7.83
S. triglyceride (mg/dl)	150.70 ± 6.34	143.20 ± 5.21	237.00 ± 18.16 ^b	225.30 ± 15.73
S. LDL (mg/dl)	92.13 ± 4.78	86.69 ± 4.64	147.10 ± 5.05 ^b	136.10 ± 5.37
S. HDL (mg/dl)	60.69 ± 4.75	60.69 ± 4.75	39.22 ± 3.79 ^c	39.22 ± 3.79

Values are mean ± SEM

Gr. 1 control, Gr. 2 control + arg, Gr. 3 unstable angina, Gr. 4 unstable angina + arg, LDL low-density lipid, HDL high-density lipid

Notes: ^a $P < 0.001$ for Gr. 1 vs. Gr. 2; ^b $P < 0.001$ for Gr. 1 vs. Gr. 3; ^c $P < 0.01$ for Gr. 1 vs. Gr. 3

Table 2 Activity of SOD, levels of total thiols and ascorbic acid in plasma of various treated groups

	Gr. 1	Gr. 2	Gr. 3	Gr. 4
SOD (unit/mg protein)	3.19 ± 0.18	3.83 ± 0.27 ^a	1.58 ± 0.13 ^b	1.64 ± 0.18
T-SH (nmol/ml)	0.49 ± 0.02	0.59 ± 0.09	0.26 ± 0.02 ^b	0.34 ± 0.03
Ascorbic acid (mg/dl)	0.65 ± 0.05	0.68 ± 0.05	0.38 ± 0.03 ^c	0.42 ± 0.04

Values are mean ± SEM

Gr. 1 control, Gr. 2 control + arg, Gr. 3 unstable angina, Gr. 4 unstable angina + arg, SOD superoxide dismutase, T-SH total thiols

Notes: ^a $P < 0.05$ for Gr. 1 vs. Gr. 2; ^b $P < 0.001$ for Gr. 1 vs. Gr. 3; ^c $P < 0.01$ for Gr. 1 vs. Gr. 3

Table 3 Activity of XO, levels of MDA and carbonyl content in plasma of various treated groups

	Gr. 1	Gr. 2	Gr. 3	Gr. 4
XO (unit/mg protein)	0.55 ± 0.03	0.34 ± 0.04 ^a	0.59 ± 0.05	0.38 ± 0.04 ^d
MDA (nmol/ml)	1.46 ± 0.08	1.12 ± 0.07 ^b	2.36 ± 0.25 ^c	1.64 ± 0.14 ^d
Carbonyl content (μmol/ml)	21.32 ± 1.82	19.30 ± 2.01	33.07 ± 3.55 ^c	21.14 ± 2.13 ^d

Values are mean ± SEM

Gr. 1 control, Gr. 2 control + arg, Gr. 3 unstable angina, Gr. 4 unstable angina + arg, XO xanthine oxidase, MDA malondialdehyde

Notes: ^a $P < 0.001$ for Gr. 1 vs. Gr. 2; ^b $P < 0.05$ for Gr. 1 vs. Gr. 2; ^c $P < 0.001$ for Gr. 1 vs. Gr. 3; ^d $P < 0.01$ for Gr. 3 vs. Gr. 4

narrow or almost blocked due to fatty deposits and plaque formation. During ischemia, insufficient oxygen supply fails to support oxidative phosphorylation resulting to lowering of ATP levels and acidosis [23]. The depletion of ATP in hypoxic tissue causes hypoxanthine and xanthine accumulation which is oxidized by xanthine oxidase leading to rapid generation of O_2 , H_2O_2 and other oxygen free radicals (OFRs) [24]. Incorporation of allopurinol, a specific inhibitor of XO, is capable of protecting the myocardium from ischemia/reperfusion induced injury and thus it is clear that the generation of OFR by XO during ischemia/reperfusion play a major role in tissue damage [25]. Free radical production during ischemia is further increased upon reperfusion [26, 27]. Oxy free radicals are removed by SOD, but during ischemia, SOD is inhibited by

excess H_2O_2 and hence fails to cope up with the increased production of free radicals and H_2O_2 resulting in excessive injury. The excess H_2O_2 , besides inhibiting SOD, causes the damage of hemoglobin heme rings and hence OH production via Fenton reaction and lipid per-oxidation [28]. Enhanced lipid-peroxidation is gauged by MDA levels and protein per-oxidation by increased carbonyl content in the plasma of patients [29]. Thiols can either chemically or enzymatically reduce dehydroascorbic acid to ascorbic acid [30]. Ascorbic acid is an effective anti-oxidant and is capable of completely protecting lipids against oxidative damage induced by free radicals, failing which increased lipid-peroxidation is observed in the patients. Increased cholesterol, triglycerides and LDL result in frequent atherosclerosis and blockage of blood vessels leading to

myocardial damage in hypercholesterolemic patients. L-arginine is a physiological precursor of nitric oxide (NO) synthesis. NO can reduce oxidative stress by inhibiting XO, scavenge superoxide radicals and terminate free radical chain reaction within lipid membrane thereby reducing inflammatory mediators [31]. Decreased ROS formation attenuates the inhibition of SOD due to which its activity increases on L-arginine supplementation. We observed that L-arginine administration decreased the carbonyl content of plasma proteins and thus reduced protein per-oxidation caused by oxidative stress. L-arginine administration to the patients was also reported to reduce the levels of serum cholesterol, triglycerides and LDL [32], but we observed significant decrease in the levels of serum cholesterol only and that too in healthy persons. May be the long term administration of L-arginine could be more effective and needs to be investigated. The noteworthy observation in the present study is that L-arginine administration significantly decreases the xanthine oxidase activity and levels of MDA and carbonyl contents in the patients. It is indicative that though L-arginine treatment does not significantly improve anti-oxidant parameters in the patients of unstable angina, it is capable of controlling free radical mediated damage by way of reducing protein and lipid per-oxidation and reducing the activity of pro-oxidant enzyme, xanthine oxidase.

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