Role of L-Arginine on Dyslipidemic Conditions of Acute Myocardial Infarction Patients

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Received: 1 November 2011 / Accepted: 15 January 2012 / Published online: 23 March 2012
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Abstract Oxidative stress conditions associated with atherosclerosis leads to oxidative modification of low-density lipoprotein (LDL). The body's capabilities to inhibit LDL oxidation and to remove or neutralize the atherogenic oxidized LDL (ox-LDL) are limited. When the LDL cholesterol level increases in the blood, it leads to dangerous consequences like atherosclerosis, leading to myocardial infarction. The major effect of an antioxidant in the LDL environment is to prevent the formation of ox-LDL (during atherogenesis. Strategies to reduce LDL oxidation and prevent atherogenesis can involve the enrichment of arterial cells with potent antioxidants that can prevent oxidative damage to the arterial wall. The objective of this study is to evaluate the effect of L-arginine on serum lipid and cholesterol levels in the patients of acute myocardial infarction (AMI). The study consisted of 70 AMI patients and 60 healthy individuals (serving as control) age 55–65 years. Serum levels of total cholesterol, high density lipoprotein (HDL), LDL and Triglycerides were determined on day 1 and day 15 of L-arginine administration (oral dose 3 g/day). The total cholesterol/HDL and the LDL/HDL ratio were calculated and compared. As per the observations, L-arginine administration was found to improve the lipid profile of the subjects. Hence it could be used as an adjuvant therapy for AMI and as a preventive measure for the onset of the disease in the healthy elderly also.

Keywords Acute myocardial infarction · Cholesterol · Cholesterol ratios · Coronary artery disease · Dyslipidemia and triglycerides

Introduction

Hyperlipidemia is regarded as an independent risk factor in the development of ischemic heart disease, and it can increase the myocardial susceptibility to ischemia/reperfusion (I/R) injury. Several previous studies have shown that enhanced serum cholesterol level is one of the important risk factors for coronary heart disease in middle aged and elderly human population [1–4]. While hyperlipidemia followed by endothelial dysfunction together serves as the root cause of atherosclerosis, progressing atherosclerosis, ulcerated plaque rupture and occlusive thrombosis lead to acute myocardial infarction (AMI). Atherosclerosis is a chronic inflammatory disease, and oxidized low-density lipoprotein (ox-LDL) is widely accepted to play an important role in atherogenesis by enhancing the intraplaque inflammatory process [5, 6]. ox-LDL is cytotoxic for endothelial cells, acts as a chemo attractant for monocytes, inhibits the motility of tissue macrophages, and triggers thrombosis by inducing platelet adhesion. All these processes accompanied by oxidative stress conditions leads to dreadful conditions.

It is widely known that L-arginine acts as the substrate for in vivo synthesis of Nitric oxide (NO) by the action of NO-synthase (NOS) enzyme. Physiological production of NO from constitutive NOS in endothelium is important to maintain cardiovascular homeostasis. Moreover, it improves microcirculation by stimulating endothelial cell proliferation and angiogenesis, inhibits SMC proliferation, decreases the expression of vascular cell adhesion molecules and

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monocyte chemotactic peptide and inhibits endothelin-1 release. NO causes myocyte relaxation and plays a vital role in the regulation of coronary circulation and myocardial contractility [7, 8]. In our previous work we found that amino acid L-arginine has a therapeutic role on oxidative stress condition during AMI. Hence it helps in reduction of oxidation of LDL cholesterol and its dire consequences [7–9]. However, the role of L-arginine in lowering the bad cholesterol during AMI is yet not very clear. So, we proposed to study the role of L-arginine in hypercholesterolemic subjects suffering from AMI.

In this study we have investigated the levels of total serum cholesterol, LDL cholesterol, high density lipoprotein (HDL) cholesterol and serum triglycerides in the healthy control subjects and patients of AMI. Further, we also calculated the ratio of non-HDL cholesterol to HDL cholesterol as a risk factor of myocardial infarction in ageing population.

Materials and Methods

The study was cleared by the departmental ethical committee. The study protocol confirms the ethical guidelines of the 1975 declaration of Helsinki. Patients from Cardiology Department, CSM Medical University and Balrampur hospital, Lucknow, UP, India were the candidates enrolled in the study. Informed consent was obtained from individuals enrolled in the study. The study included 70 patients of the age group between 40 and 75 suffering from AMI and 60 healthy subjects of the same age group. Patients hospitalized with confirmed clinical diagnosis of myocardial infarction before reperfusion therapy were included in the AMI group of the study. These patients had increased levels of CPK-MB and Troponin-T. All these patients were receiving regular antihypertensive medication. 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 in the following way:

Group (1): included 60 healthy subjects (male/female = 48/12) age 60 ± 2.5 years, weight 62 ± 2.4 kg, systolic blood pressure 117 ± 3 mmHg, diastolic blood pressure 85 ± 4 mmHg, without any previous disease record who served as control.

Group (2): comprised of 55 healthy subjects (male/female = 43/12) from group 1, supplemented with oral dose of L-arginine (3 g/day) for 15 days. These subjects weighted 61 ± 1.6 kg, systolic blood pressure 110 ± 2 mmHg, diastolic blood pressure 78 ± 3 mmHg after L-arginine supplementation for 15 days.

Group (3): included 70 AMI patients (male/female = 43/27) age 63 ± 3.9 years, weight 75.2 ± 5.9 kg, systolic blood pressure 157 ± 4 mmHg, diastolic blood pressure 140 ± 3 mmHg, receiving regular therapy antihypertensive drugs like nitroglycerine, montrate, metoprolol, aspirin and clopidogrel.

Group (4): included 60 MI patients (male/female = 40/20) from group 3, assigned to regular therapy along with supplementation of oral dose of L-arginine (3 g/day) for 15 days. These subjects weighted 72.5 ± 3.8 kg, systolic blood pressure 140 ± 3 mmHg, diastolic blood pressure 86 ± 4 mmHg after L-arginine supplementation for 15 days.

All the chemicals employed in the study were AnalR 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 and Merrell [10] and Adams et al. [11]. Three grams of powdered L-arginine (Sigma Co., USA) in capsule form were provided to the subjects. 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, hence 3 g/day of L-arginine was selected as an optimum dose.

Biochemical Analysis

Serum level of triglyceride was measured by standard protocol of cholesterol oxidase/peroxidase phosphotungstate method (Kit supplied by Span Diagnostic Ltd.; Surat; India). The absorbance was noted at 520 nm and the Triglyceride level in serum was expressed in mg/dl. Serum Total Cholesterol was quantified by spectrophotometer method by the addition of enzyme present in the reagent kit. The absorbance of red quinone imine complex was determined at 505 nm. The value of Total cholesterol present in serum was expressed in mg/dl.

Serum LDL cholesterol was calculated by Friedwald equation as follows:

[VLDL cholesterol = serum triglyceride/5; HDL cholesterol was measured biochemically]

LDL cholesterol = serum total cholesterol – VLDL cholesterol – HDL cholesterol. Results were expressed in mg/dl.

Statistical Analysis

The results are expressed as mean $\pm$ SE. One way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison test has been applied to test the significance of the data. The p value <0.05 was considered to be statistically significant.

Results

The results obtained as per the study and the levels of significance of the data are shown in the table. As observed in the table provided, L-arginine supplementation lead to a significant reduction in the total serum cholesterol levels of the control and patient groups. The LDL cholesterol and serum triglyceride also decreased while the HDL cholesterol showed significant elevation after the therapy. The total cholesterol level reduced significantly in the C + Arg and AMI + Arg groups when compared to the Control and the AMI groups ($p < 0.01$; C + Arg vs C and $p < 0.001$; AMI + Arg vs AMI). Similarly the LDL cholesterol level also reduced significantly on L-arginine therapy to the subjects ($p < 0.001$; C + Arg vs C and $p < 0.01$; AMI + Arg vs AMI). Triglyceride levels reduced but not significantly in the AMI + Arg group when compared to the AMI group, while HDL cholesterol showed significant elevation upon L-arginine supplementation to the subjects ($p < 0.05$; C + Arg vs C and $p < 0.001$; AMI + Arg vs AMI) (Table 1). The total cholesterol to HDL cholesterol ratio after 15 day L-arginine supplementation reduced significant in both the control ($p < 0.05$) and patient ($p < 0.01$) groups. The LDL cholesterol to HDL cholesterol ratio also decreased significantly in control ($p < 0.01$) and patient ($p < 0.05$) groups (Table 1). These observations emphasize the fact that the AMI patients in this study were suffering from dyslipidemia and L-arginine supplementation lead to an improvement of this dyslipidemic condition. Since L-arginine administration to patients is very effective in reducing the bad cholesterol level, hence is helpful to overcome the dyslipidemic condition during AMI.

Discussion

Myocardial ischemia results from inadequate supply to the myocardium. During ischemia, insufficient oxygen supply

fails to support oxidative phosphorylation resulting to lowering of ATP levels and acidosis [6]. 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) [12, 13]. Excessive free radical generation during ischemia leads to frequent oxidation of serum LDL which further plays a significant role in atherogenesis and its terrible consequences. Endothelial dysfunction creates a local milieu which facilitates the initiation and development of atherogenic process. Circulating leukocytes, predominantly monocytes are attracted and bind to activated endothelial cells followed by migration into the sub endothelial layer where they transform into macrophages. Here they act as local scavenger cells with the capacity to take up modified LDL cholesterol and triglycerides ultimately becoming the characteristic foam cell of atherosclerosis. These early lesions are called fatty streaks. These lesions develop into fibrous plaques as a consequence of further lipid accumulation [6–9]. Progressive plaque accumulation results in luminal narrowing and ultimately vessel obstruction leading to myocardial infarction and sometimes sudden cardiac arrest and death. L-arginine has an indirect antioxidant role and is a naturally occurring continuously released vasoactive agent that controls coronary vascular tone [9]. NO is synthesized by the vascular endothelium through the conversion of L-arginine to L-citrulline by NOS [8]. It has been found to be cardioprotective in I/R [14] and exerts direct effect on cardiac myocytes. L-arginine through NO has an indirect antioxidant role to scavenge OFRs and inhibit XO activity, thus reducing ROS formation. Reduced ROS formation subsequently attenuates the oxidation of lipids. In our previous works [6] we observed that L-arginine reduces oxidative stress and scavenges superoxide radicals. Several other studies have shown that L-arginine have a vasomotor effect through the production of endothelial derived relaxing factor i.e. NO [15–18]. Also, L-arginine reduces endothelial

Table 1 Serum cholesterol levels of the subjects in the study

	Control (C) (day 1)	C + arginine (15th day)	AMI (day 1)	AMI + arginine (15th day)
Total serum Chl (mg/dl)	170.40 ± 1.25	148.63 ± 1.06***	220 ± 2.15	149.93 ± 1.24****
S. HDL Chl (mg/dl)	52.16 ± 1.43 [@]	55.23 ± 0.86	31.73 ± 1.98 ^{@@}	41.7 ± 0.57
S. LDL Chl (mg/dl)	99.86 ± 0.73	79.34 ± 0.98*	134.08 ± 2.10	182.4 ± 1.00**
Triglycerides (mg/dl)	79.81 ± 1.69	63.26 ± 1.24 [#]	143.26 ± 0.75	119.34 ± 1.01
Total Chl/HDL Chl (mg/dl)	3.25	2.54 ^a	6.72	3.69 ^b
LDL Chl/HDL Chl (mg/dl)	1.94	1.47 ^c	4.11	2.65 ^d

Values are presented as mean ± SE

AMI acute myocardial infarction, Chl cholesterol

*** $p < 0.01$ versus C group, **** $p < 0.001$ versus AMI group, [@] $p < 0.05$ versus C + Arg, ^{@@} $p < 0.001$ versus AMI + Arg, * $p < 0.001$ versus C group, ** $p < 0.001$ versus AMI group, [#] $p < 0.05$ versus C group, ^a $p < 0.05$ versus C, ^b $p < 0.01$ versus C, ^c $p < 0.01$ versus AMI, ^d $p < 0.05$ versus AMI

inflammation and myocardial stunning during I/R injury [19]. In this present work we find that L-arginine actively reduces bad cholesterol and thus plays a vital role in attenuating the damage being caused due to oxidation of lipids during AMI. Our findings are supported by some earlier workers [15–20].

Conclusion

The present study shows that L-arginine administration plays a significant role in reducing the modified serum cholesterol levels in patients of AMI and promotes the elevation of HDL cholesterol. Also, as evident from the results, L-arginine could be used as an adjuvant therapy in healthy elderly population as a preventive measure against precipitation of myocardial ischemia without any side effect as L-arginine is a naturally occurring conditionally essential amino acid to human beings.

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