ANTIOXIDANT EFFECTS OF L-SERINE AGAINST FATTY STREAK FORMATION IN HYPERCHOLESTEROLEMIC ANIMALS

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Abstract

INTRODUCTION: Peroxidation of blood lipoproteins is regarded as a key event in the development of atherosclerosis. Evidence suggests that oxidative modification of amino acids in low-density lipoprotein (LDL) particles leads to its convert into an atherogenic form, which is taken up by macrophages. Therefore the reduction of oxidative modification of lipoproteins by increasing plasma antioxidant capacity may prevent cardiovascular disease.

METHODS: In this study, the antioxidant and anti-fatty streak effects of L-serine were investigated in hypercholesterolemic rabbits. Rabbits were randomly divided into three groups which were fed high-cholesterol diet (hypercholesterolemic control group), high-cholesterol + L-serine diet (treatment group), and normal diet (control) for twelve weeks and then blood samples were obtained to measure plasma cholesterol, triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), antioxidant capacity (AC), malondialdehyde (MDA), and conjugated dienes (CDS). Right and left coronary arteries were also obtained for histological evaluation.

RESULTS: No significant difference was observed in plasma cholesterol, TG, HDL, LDL and CDS levels between treatment and hypercholesterolemic control groups ($P>0.05$). The levels of plasma MDA and AC were 0.29 μM and 56%, respectively in the treatment group which showed a significant change in comparison with hypercholesterolemic control groups ($P<0.05$). The mean size of produced fatty streak also showed significant reduction in the treatment group compared to the hypercholesterolemic group ($P<0.05$).

CONCLUSIONS: The results showed that L-serine has antioxidant and anti-fatty streak effects without any influence on plasma lipid levels in hypercholesterolemic rabbits.

Keywords: Atherosclerosis, cholesterol, L-serine, antioxidant, lipids, fatty streak.

ARYA Atherosclerosis Journal 2006, 2(3): 126-129

Introduction

Hypercholesterolemia is one of the most important risk factors of ischemic heart disease.[1]

Hypercholesterolemia damages the vascular endothelium and results in impaired endothelium-dependent vasomotor function, which is improved by lipid-lowering therapy. Oxidative stress appears to play an important role in mediating the effects of hypercholesterolemia and recent studies have investigated the effects of antioxidants on endothelial function in patients with hypercholesterolemia.[2]

Oxidative modification of LDL is a key event in early atherogenesis, with oxidized LDL (ox-LDL) contributing to the accumulation of cholesterol and oxidized lipids in the arterial wall.[3,4]

Antioxidants reduce the oxidation of LDL and decrease the concentration of free radicals, which inactivate nitric oxide and may therefore be effective in reversing endothelial function associated with hypercholesterolemia.[5]

Serum is a potent antioxidant and can protect LDL against oxidation by macrophages.[6] Amino acids, e.g. cysteine, histidine, arginine and tyrosine can protect LDL against oxidation.[7-10] Plasma may thus protect LDL from oxidation in the circulation and this may help to explain why extensively oxidized LDL is probably not formed in the blood stream. This study was designed to analyze the antioxidative effect of L-serine against fatty streak formation in cholesterol-fed rabbits.

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Date of submission: August 11, 2006
Date of acceptance: November 23, 2006

Materials and methods

Male New Zealand white rabbits weighing 1.5 kg were obtained from the central animal house of Tehran Pasteur Institute. The animals were housed at the temperature of 21-23 °C in a light-controlled room with a 12-hour light-dark cycle and ambient humidity (50-60%). All the rabbits were initially fed a normal diet (Pars Dam, Tehran, Iran) for 2 weeks and then randomly divided into a hypercholesterolemic control group (n=5, fed 1% high-cholesterol diet) and treatment group (n=5, fed 1% high-cholesterol diet supplemented with L-serine) and rabbits fed a normal diet were used as normal controls. Animals were fed for 12 weeks and each diet was set at 100 g/rabbit per day with water available ad libitum. The rabbits were weighed weekly. Blood samples from a superficial ear vein were taken at 0, 6 and 12 weeks after 12 hours of fasting to analyze plasma total cholesterol, LDL cholesterol and HDL cholesterol and triglyceride by use of commercially available spectrophotometric assay kits (Randox, England). Plasma antioxidant capacity (AC), malondialdehyde (MDA) and conjugated dienes (CDS) were determined by using the spectrophotometric method.[11-13] The animals were sacrificed and the right and left coronary arteries were dissected and fixed in formalin for pathological examination. The histological sections were stained with hematoxylin/eosin. The morphometric measurement of fatty streak formation was carried out by oculometer (Micrometer Scale) and studied under light microscope.[14] All values are given as means ± SEM. Statistical significance was tested by use of ANOVA for repeated measures followed by Scheffe's F test.

Results

Plasma total cholesterol (baseline: 235±17 mg/dl) increased to 2400±351 mg/dl after 12 weeks of the 1% cholesterol-enriched diet (Table 1).

Dietary L-serine had no significant effect on the plasma cholesterol level in the L-serine-treated group. Similar changes were also observed for plasma LDL cholesterol, whereas HDL cholesterol levels were not significantly different between the groups (Table 1).

Plasma AC (baseline: 75±4%) decreased to 23±4% and plasma MDA increased 3.4 fold in the hypercholesterolemic controls compared to normal controls. Dietary L-serine had a significant effect on plasma AC and MDA; AC increased by 143 % and MDA decreased by 52% in the L-serine-treated group compared to the hypercholesterolemic control group (Table 1).

Pathological assessment of right and left coronary arteries showed that rabbits which had received a normal diet (Table 2, Figure 1a) and those which had received a high-cholesterol diet supplemented with L-serine did not develop any fatty streaks (Table 2, Figure 1c), whereas the rabbits which had received a high-cholesterol diet showed fatty streak formation (Table 2, Figure 1b).

Discussion

Hypercholesterolemia and the subsequent atherosclerosis is one of the most important risk factors for ischemic heart disease.

Atherosclerosis is characterized by accumulation of intra- and extracellular lipids, monocyte/macrophage infiltration, and foam cell formation of connective tissue components.

TABLE 1. Effect of L-serine on plasma lipids, MDA and AC in cholesterol-fed rabbits at 12 weeks**

Group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL cholesterol (mg/dl)	HDL cholesterol (mg/dl)	MDA (μM)	AC RBC lysis (%)
Control	235± 17	170± 15	107± 18	39± 3	0.34±0.13	75±4
Hypercholesterolemic	2400± 351	400± 83	1261± 239	58± 7	1.14±0.15*	23±4*
L-Serine-treated	2217± 391	405± 50	1097± 179	43± 3	0.55±0.14*	56±3*

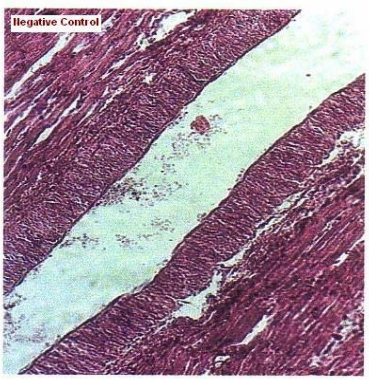
* Data in columns were significantly different at P< 0.05
** Values are means ± SEM (n = 5)

TABLE 2. Pathological evaluation of right and left coronary arteries in cholesterol fed rabbits**

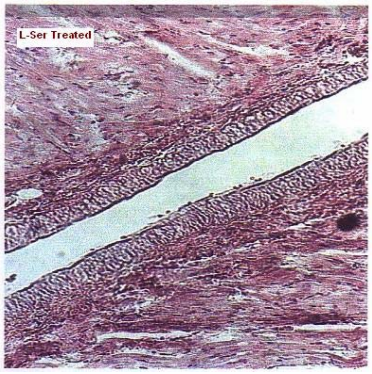
Group	Diet	Right coronary artery (μ)	Left coronary artery (μ)
Control	Normal diet	0±0	0±0
Hypercholesterolemic	High-cholesterol diet	1.8± 0.3*	2.6± 0.8*
L-serine-treated	High-cholesterol diet + L-serine	0±0*	0±0*

* Data in columns were significantly different at P<0.05
** Values (relative size of plaque) are means ± SEM (n=5)

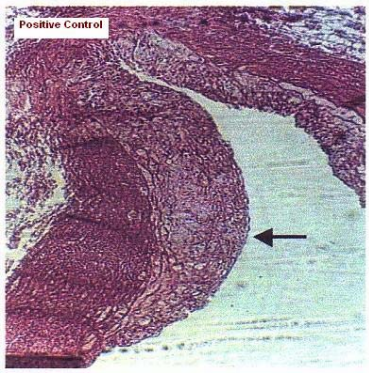
FIGURE 1. Pathological evaluation of coronary arteries in all groups



a: Left coronary artery of normal control (magnification: 100×)



b: Left coronary artery of hypercholesterolemic control (magnification: 100×)



c: Left coronary artery of L-Ser treated group (magnification: 100×)

Based on experimental studies, ox-LDL has a key role, which could be involved in many of the above-mentioned features of atherogenesis. Antioxidant therapy and inhibition of LDL peroxidation might yield beneficial results in this context. Our previous study showed that oxidative hemolysis of erythrocytes and MDA produced from isolated hepatocytes decreased in presence of various concentrations of L-serine. In our study, the elevation in total serum cholesterol, LDL and HDL upon cholesterol feeding is not surprising and is in agreement with several studies.[15,16] In contrast, a significant decreases in plasma HDL in cholesterol-fed rabbits was reported by Tsai and Chen.[17] However, in our study the dramatic increase in total serum cholesterol seemed to have mainly originated from the remarkable increase in LDL rather than HDL. Dietary L-serine had no significant effect on the plasma cholesterol level in hypercholesterolemic rabbis. Similar changes were also observed for plasma LDL, whereas HDL levels were not significantly different between the groups. Fatty streak formation in coronary arteries was induced by hypercholesterolemia in the high-cholesterol diet group.

Our results demonstrated that L-serine treatment significantly inhibited fatty streak formation in arteries compared with high-cholesterol diet group without any significant effect on plasma concentrations of lipids. L-serine treatment also had significant effect on plasma AC and MDA, hence plasma AC increased and plasma MDA decreased in atherosclerotic rabbits. Our findings suggest that L-serine can prevent fatty streak formation in hypercholesterolemic rabbits, possibly through increasing antioxidant capacity and reduction of lipid peroxidation.

Further studies are needed to evaluate the mechanism(s) underlying the antiatherogenic effect of L-serine in hypercholesterolemic rabbits.

Acknowledgements

This work was supported by the Research Council of Isfahan University of Medical Sciences and Isfahan Pharmaceutical Research Center.

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