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Comparison of lycopene and fluvastatin effects on atherosclerosis induced by a high-fat diet in rabbits

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Abstract

Objective: We evaluated the antiatherogenic effect of lycopene in rabbits fed a high-fat diet.

Methods: Forty adult male rabbits were divided into five groups that were fed a standard diet, a high-fat diet, a high-fat diet plus 4 mg/kg of lycopene, a high-fat diet plus 12 mg/kg of lycopene, and a high-fat diet plus 10 mg/kg of fluvastatin, respectively. Lycopene and fluvastatin were administered intragastrically. The level of serum total cholesterol, total triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total antioxidant capacity, and malondialdehyde were measured before and after 4 and 8 wk of experimental treatment. In addition, plasma levels of lycopene, oxidized low-density lipoprotein, serum nitric oxide, and interleukin-1 were measured after the experiment. The area of atherosclerotic plaque and pathologic changes of the aorta were evaluated.

Results: Compared with the control, levels of total cholesterol, total triacylglycerol, low-density lipoprotein cholesterol, malonaldehyde, oxidized low-density lipoprotein, and interleukin-1 were increased and total antioxidant capacity and nitric oxide were decreased in the animals with a high-fat diet ($P < 0.05$). Intragastric administration of lycopene counteracted the change in these parameters ($P < 0.05$). In this case, the data showed that lycopene in the used dose was better than the fluvastatin intervention. Morphologic analysis revealed that lycopene and fluvastatin markedly reduced the formation of atherosclerotic plaques in the aorta compared with the situation in rabbits on a high-fat diet alone.

Conclusion: Lycopene, like fluvastatin, significantly attenuated atherogenesis in rabbits fed a high-fat diet.

Keywords: Lycopene; Fluvastatin; Atherogenesis; Rabbit

Introduction

Atherosclerosis is one of the most widespread conditions that threaten human health and survival [1–3]. The basic pathogenesis of atherosclerosis involves an insult to the endothelial and smooth muscle cells of the arterial wall by various harmful factors such as viral infection, mechanical damage, and dyslipidemia, especially abnormal oxidized low-density lipoprotein (ox-LDL), leading to an excessive chronic inflammatory/fibroproliferative response. The pathologic process results in a progressive accumulation of lipids and fibrous elements in the large arteries [1–4]. Based on the effect of oxidation and modification of LDL on the development of atherosclerosis, it might be possible to prevent the initiation and progression of lesions by therapy with antioxidants. Recent investigations have shown that all categories of high-risk patients benefit from LDL-lowering therapy with statins [5–7]. Fluvastatin, one of the generally accepted drugs for treatment of coronary atherosclerosis, bases its effects on antioxidation and suppression of LDL levels, but not without sizable costs and risk for side effects [8]. Thus, it would be valuable to search for substances or ingredients with antioxidative effects in our daily food.

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Lycopene is an open-chain unsaturated carotenoid reported to be an efficient antioxidant. It is the pigment molecule that imparts the red color to some fruits and is found in high concentration in tomatoes and tomato products, watermelons, red grapefruits, and guava [9–14]. Epidemiologic investigations have reported that lycopene consumption can reduce and prevent the development of cardiovascular disease [15–20]. Experimental studies have shown that it has many important bioactivities, e.g., quenching singlet oxygen, eliminating reactive oxygen species, blocking lipid peroxidation, suppressing cell reproduction, reinforcing immunity, and inducing gap junction intercellular communication [10,12–14]. It is one of the most popular research topics in nutrition and pharmaceutics [12,20,21]. However, few quantitative experimental studies of the preventive effect of lycopene on atherosclerosis have been reported, especially in vivo. The present study was designed to elucidate the effects of lycopene on the development of atherosclerosis in rabbits on a high-fat diet. Comparing the results with the antiatherosclerotic effect of fluvastatin would provide laboratory evidence for the potential of lycopene as a therapeutic agent in atherosclerosis.

Materials and methods

Composition of diets

The standard diet for rabbits consisted of 10% wheat, 40% grass powder, 12% soybean cake, 20% corn, 10% wheat bran, 3% fish flour, 1% salt, 3% bone meal, and 1% multivitamins (percent weight). The diet contained 3.5% fat, 20% protein, and 25% carbohydrates and was purchased readymade from the Department of Zoology, Xiangya School of Medicine, Central South University. The high-fat experimental diet consisted of 5% lard, 1% cholesterol, and 94% standard diet.

Pharmaceuticals and reagents

Lycopene was purchased from the Huabei Pharmaceuticals Limited Company (Shijiazhuang, China; purity 90%, batch no. 041202). The standard lycopene preparation was purchased from Sigma Chemical Company (St. Louis, MO, USA; purity 95%). The fluvastatin-sodium capsule was purchased from the Nuohua Pharmaceuticals Limited Company (Beijing, China; batch no. X0006). Cholesterol was a product from the Tongxin Biochemistry Factory (Qidong, Hunan, China; batch no 050422). Total cholesterol (TC), total triacylglycerol (TG), LDL cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) kits were purchased from Dongou Biological Engineering Limited Company (Wenzhou, Zhejiang, China). Total antioxidant capacity (T-AOC), malonaldehyde (MDA), and nitric oxide (NO) kits were obtained from Jiancheng Biological Engineering Institution (Nanjing, China). Ox-LDL and interleukin-1 (IL-1) enzyme-linked immune assay reagent kits were purchased from Dingguo Biology Limited Company (Shanghai, China).

Experimental design

Forty male New Zealand white rabbits with a body weight of 1800 ± 300 g were used in this study. After 1 wk of adaptation to the standard diet, blood samples were collected from the rabbit ear artery to measure basal lipid levels.

Based on the obtained level of TC, the rabbits were stratified into five groups of eight animals with similar distributions of pretreatment cholesterol levels. Group C (normal control) was fed the standard diet during the experimental period; group F received the high-fat diet; group FL1 (low-dose lycopene) the high-fat diet plus 4 mg of lycopene $\cdot$ $kg^{-1} \cdot d^{-1}$; group FL2 (high-dose lycopene) the high-fat diet plus 12 mg of lycopene $\cdot$ $kg^{-1} \cdot d^{-1}$; group FF (fluvastatin) the high-fat diet plus 10 mg of fluvastatin $\cdot$ $kg^{-1} \cdot d^{-1}$. Both drugs were dissolved in 1% sodium carboxymethyl cellulose to a final solvent volume of 2 mL/kg of body weight. The drugs were administered intragastrically, once per day, by a 12-Fr catheter inserted into the stomach. Groups C and F received the same amount of solvent without the drugs.

The duration of treatment was 8 wk for each animal. They were kept two together in each cage with daylight illumination and access to water ad libitum. The food intake was recorded everyday and, based on the consumption of normal rabbits in a pilot experiment, restricted to 150 ± 10 g of the compositions above. The temperature in the animal room was controlled at 18 ± 4°C, and humidity was kept at 60–65%. The general health and activity of rabbits were monitored closely. All experimental procedures were conducted in accordance with the guidelines of the animal ethical committee for animal experimentation in China.

Assessment of plasma lycopene

The lycopene standard solution was prepared by dissolving 1 mg of standard lycopene in 5 mL of isopropyl alcohol (0.2 mg/mL). This solution was further diluted to final concentrations of 2, 1, 0.5, 0.25, 0.125, 0.063, 0.031, 0.016, 0.008, and 0.004 μg/mL to obtain standard lycopene high-performance liquid chromatographic curves. The area under the lycopene peak was then plotted against the corresponding concentration, revealing good linearity in the range of interest ($y = -0.0006 + 8.2E - 7x$, $R^2 = 0.9997$). To determine plasma lycopene concentration, 400 μL of rabbit plasma was mixed with 200 μL of ethanol and vortex-mixed for 20 min; 500 μL of water was added followed by 500 μL of *N*-hexane and the mixture was vortex-mixed for another minute. After centrifugation for 10 min at 1900 × *g*, the upper phase was removed and, by adding 1 mL of *N*-hexane, a second upper phase was obtained. Both extracts