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Basic nutritional investigation

Downregulation of hepatic lipoprotein assembly in rats by fermented products of *Monascus pilosus*

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Abstract

Objective: Hypercholesterolemia is a major risk factor for atherosclerosis. The fermented products of *Monascus* sp. have been known for their antihypercholesterolemic effect; however, the studies mostly have focused on the inhibition of cholesterol biosynthesis in liver. In this study, we examined whether fermented products of *Monascus pilosus* have regulatory effects on the hepatic lipoprotein assembly.

Methods: Male Wistar rats were fed 1% cholesterol diet for 2 wk. After hypercholesterolemia was induced, the animals were maintained on this cholesterol diet supplemented with *M. pilosus*–fermented products grown in regular medium/garlic-containing medium or garlic powder for another 6 wk. The concentration of blood lipids and the expression of proteins involved in lipoprotein assembly and hepatic antioxidation were assayed.

Results: Maintenance on a 1% cholesterol diet for 2 wk significantly ($P < 0.05$) raised animals' blood lipid levels and increased the expression of intestinal microsomal triacylglycerol transfer protein, hepatic apolipoprotein B-100, and acyl-coenzyme A:cholesterol acyltransferase. Supplementation of *M. pilosus*–fermented products or garlic powder significantly ($P < 0.05$) lowered animals' blood lipid levels and inhibited the expression of intestinal microsomal triacylglycerol transfer protein and hepatic apolipoprotein B-100. The 3-hydroxy-3-methylglutaryl-coenzyme A reductase was downregulated by the *M. pilosus*–fermented product grown in regular medium but not in garlic-containing medium. The expression of antioxidant enzymes was significantly upregulated by the *M. pilosus*–fermented product grown in garlic-containing medium.

Conclusion: *Monascus* sp.–fermented products exert the hypocholesterolemic effect by mechanisms other than the inhibition of cholesterol biosynthesis.

Keywords: *Monascus pilosus*; Garlic; Microsomal triacylglycerol transfer protein; Apolipoprotein B-100; Acyl-coenzyme A:cholesterol acyltransferase

Introduction

Atherosclerosis is the leading cause of death in modern societies. Elevation in blood lipids, especially low-density lipoprotein (LDL) cholesterol, is one important risk factor for atherosclerosis. LDL oxidation has been recognized as having a crucial role in the initiation and progression of atherosclerosis [1]. In vitro and in vivo data have shown that oxidation of LDL contributes to the etiology of atherosclerosis [2]. Antioxidants that prevent LDL oxidation in vitro also inhibit atherosclerosis in animal models [3,4]. Therefore, reduction of blood lipids and inhibition of lipid oxidation are considered critical to the prevention/alleviation of atherosclerosis.

Supplementation of nutraceuticals to reduce blood lipids and lipid oxidation has become more common in Asia in recent years. Commercial *Monascus* sp.–fermented products are nutraceuticals that are widely used for the prevention of hypercholesterolemia. *Monascus* belongs to the class Ascomycetes and the family Monascaceae [5], known as producers of various secondary metabolites with polyketide structures. Metabolites produced by *Monascus* sp. fermentation has been applied in the processing of various foods such as rice (red mold rice, also named *anka*), meat (red mold meat), soybean curd (*tofuyo*), and vegetables in Asia

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for hundreds of years [6]. Monacolin K, one of the secondary metabolites from *Monascus* sp. fermentation, was recognized as a potent 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor [7]. Studies have indicated that long-term intake of foods containing monacolin K or compounds with similar structure significantly lowers plasma cholesterol concentration [8–10]. In addition to the blood lipid-lowering effect, *Monascus* sp.–fermented products exert antioxidant activities [11]; however, these activities are not comparable to those of many plant foods. In a previous study, we raised the antioxidant activities of *Monascus pilosus*–fermented products by supplementing garlic, a potent antioxidant, into culture medium [12], making *Monascus* sp. products more nutraceutically valuable for antioxidation.

There are many promising lines of research suggesting the antihypercholesterolemic effect of *Monascus* sp. fermented products; however, the studies mostly have focused on the inhibition of cholesterol biosynthesis in the liver. The concentration of blood lipids is influenced by the assembly of very–low-density-lipoprotein (VLDL) in the liver; therefore, the goal of this study was to examine the regulatory effects of *M. pilosus*–fermented products grown in regular and garlic-containing media on the expression of proteins involved in VLDL assembly in cholesterol-fed rats. The results of this study will help to elucidate the mechanisms underlying the alleviation of hypercholesterolemia by *Monascus* sp.–fermented products.

Materials and methods

Preparation of fermented products

Monascus pilosus BCRC 31527 (Biosource Collection and Research Center of Food Industry Research and Development Institute, Shin Chu, Taiwan) was fermented by the method of Kuo et al. [12] to produce "MP" powder (mycelia extract of *M. pilosus*–submerged culture) and "GMP" powder (mycelia extract of *M. pilosus*–submerged garlic-containing culture). Garlic powder was also prepared as described in the study by Kuo et al. [12].

Animal and diets

All animal experiments reported herein were approved by the Shih Chien University animal care and use committee. Twelve-week-old male Wister rats were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The animals were housed in stainless steel cages at 22 ± 2°C and l2-h light/dark cycle and had free access to water and ground Purina rat chow (Ralston Purina, St. Louis, MO, USA). After a week of acclimation, the animals were fed a basal diet for 2 wk before receiving a high-cholesterol diet (HCD; 1% cholesterol, 5% lard, 5% soybean oil, 20% casein, 35.1% corn starch, 20% sucrose, 3.5% AIN-76 minerals, 1% AIN-76 vitamins, 0.4% choline, 9% methyl α-cellulose). Two weeks after switching to the HCD, 10 rats from the basal group and from the high-cholesterol group were sacrificed to ensure the significant increase in blood lipids.

After maintenance on the HCD for 2 wk, animals were divided into four groups: group HCD, group GA (HCD supplemented with 2.5% garlic powder [GA]), group MP (HCD supplemented with 2.5% MP powder), and group GMP (HCD supplemented with 2.5% GMP powder). The animals were kept on the diets for 6 wk.

Determination of serum and liver lipids

Serum triacylglycerol, total cholesterol, LDL cholesterol, and high-density lipoprotein cholesterol were determined by using commercially available kits (Randox, Antrim, United Kingdom). Liver cholesterol was measured the same way as serum after extracting lipids from the liver according to the method of Folch et al. [13].

Preparation of tissue microsomes

Tissues for analysis were thawed on ice and homogenized in 25 mM Tris-HCl, pH 7.4, containing 250 mM sucrose and 1 mM ethylene-diaminetetra-acetic acid (10%, w/v). The homogenates were first centrifuged at 10 000 × *g* for 30 min, and then the supernatant was further centrifuged at 105 000 × *g* for 60 min to separate cytosol from microsomes. The microsome pellets were resuspended in 25 mM Tris-HCl, pH 7.4, containing 10% glycerin (w/v) and 1 mM ethylene-diaminetetra-acetic acid.

Immunoblot analysis

Microsomes (20 μg) prepared from rat livers and small intestines were added to 20 μL of sample buffer (30 mM Tris-HCl, 1% sodium dodecylsulfate, 0.1 M sucrose, 8 M urea, 5% β-mercaptoethanol, 0.005% bromophenol blue, pH 6.8) and boiled for 5 min before being subjected to gel electrophoresis on a 8% sodium dodecylsulfate/polyacrylamide gel. The separated proteins were transferred electrophoretically to a polyvinylidene diflouride membrane (GE Healthcare, Pittsburg, PA, USA). The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline with Tween, 20 mM Tris-HCl, pH 8.3, 137 mM NaCl, and 0.1% Tween 20 for 1 h and then incubated at room temperature with 1:3000 dilution of anti–microsomal triacylglycerol transfer protein (MTP) polyclonal antibody (BD Biosciences, Palo Alto, CA, USA), 1:500 dilution of anti–apolipoprotein B-100 (apoB-100) polyclonal antibody (Biodesign, Saco, ME, USA), 1:500 dilution of anti–acyl-CoA:cholesterol acyltransferase (ACAT) polyclonal antibody (Santa Cruz, Santa Cruz, CA, USA), 1:500 dilution of anti–HMG-CoA reductase polyclonal antibody (Upstate, Charlottesville, VA, USA), 1:5000 dilution of anti–copper/zinc superoxide dismutase (SOD) polyclonal antibody, 1:4000 di-