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Basic nutritional investigation

Lipoic acid prevents high-fat diet–induced dyslipidemia and oxidative stress: A microarray analysis

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Abstract

Objective: We previously found that lipoic acid (LA) improved high-fat diet (HFD)–induced dyslipidemia in rats. To elucidate the molecular mechanisms of that effect, we carried out experiments aimed at analyzing biochemical parameters and gene expression profiles.

Methods: C57BL/6 mice were randomly assigned to one of three groups ($n = 8$). The control group consumed an ordinary diet (4.89% fat, w/w). The other two experimental groups were fed with an HFD (21.45% fat, w/w) or an HFD plus 0.1% LA. After 6 wk, plasma lipid level and antioxidant status were examined. To investigate the molecular mechanisms underlying the effects of LA on lipid metabolism and oxidative stress, we examined gene expression profiles in liver using the GeneChip microarray system. The differential expression of genes of interest identified by microarray technique was validated by real-time reverse transcription–polymerase chain reaction.

Results: HFD resulted in significant alterations in lipid profiles and a depressed antioxidant defense system. LA supplementation induced decreases in lipid peroxidation, plasma cholesterol, triacylglycerols, and low-density lipoprotein cholesterol and an increase in high-density lipoprotein in HFD-fed mice. DNA microarray analysis of the liver showed that LA ingestion upregulated the expression of genes related to β-oxidation and free radical scavenger enzymes, whereas those involved in cholesterol synthesis were downregulated.

Conclusion: LA can prevent HFD-induced dyslipidemia by modulating lipid metabolism, especially by increasing β-oxidation and decreasing cholesterol synthesis, and oxidative stress by increasing those of free radical scavenger enzyme gene expression.

Keywords: DNA microarray; Hyperlipidemia; Oxidative stress; Lipid metabolism; High-fat diet

Introduction

A high-fat diet (HFD) has been reported to adversely affect the health of humans and animal species [1–4]. The prevalence of dyslipidemia resulting from excess energy intake and physical inactivity is increasing in China. Abnormal lipid metabolism is a main cause of dyslipidemia, which is a major risk factor for cardiovascular disease, obesity, cholestasis, and overall mortality [5]. It has been reported that high levels of fat increase fat-mediated oxidative stress and decrease antioxidative enzyme activity [6]. In contrast, there are various reports indicating the beneficial effects of antioxidant supplementation in preventing dyslipidemia and cardiovascular disease [7–9]. Thus, oxidative damage and its consequences may result in many chronic health problems that are attributed to an HFD. The liver plays a central role in the maintenance of systemic lipid homeostasis and is especially susceptible to reactive oxygen species (ROSs) damage [10]. This organ supplies energy substrates to peripheral tissues by the Cori cycle and glycogen catabolism and is important for detoxification. Oxidative stress–related factors could be implicated in the functional impairment of the liver, associated with an exacerbated nutrient oxidation. ROSs have detrimental effects on hepatocytes by damaging DNA, lipids, and proteins, leading to a disruption in cellular homeostasis and aggravating metabolic syndrome features [11,12].

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Lipoic acid (LA) not only scavenges free radicals directly but also provides the reducing medium for the regeneration of the antioxidant from its oxidized form. It has been recognized as a universal antioxidant [13]. Although we previously found that LA is effective in the prevention of dyslipidemia, the mechanisms by which this antioxidant controls lipid metabolism have not been elucidated. In this study, we verified the antihyperlipidemic effects of LA and examined the gene expression profiles in mouse liver by using the GeneChip microarray system to reveal the molecular mechanisms underlying the effects of LA on lipid metabolism and oxidative stress.

Materials and methods

Animals

The experiment was conducted with male C57BL/6 mice (4 wk old, 14.92 ± 0.97 g). The animals were housed under conditions of controlled temperature (23 ± 2°C) and humidity (60%) with natural light. The experimental protocol was developed according to the institution's guideline for the care and use of laboratory animals.

Experimental design

Test animals were fed initially, before the study, standard diets for 1 wk for adaptation. Then they were assigned to one of three groups with eight mice in each group. Group I (control) received only a normal diet containing 4.89% fat. Group II (HFD) received an HFD containing 21.45% fat. Group III (LA) was fed with the HFD in conjunction with 0.1% LA. Compositions of the animal diets are listed in Table 1. All mice were allowed free access to the test diets and deionized water throughout the test period. At the end of the experimental periods, mice were deprived of food for 12 h and then they were slightly anesthetized and sacrificed by decapitation. Blood was collected from the neck into glass tubes. Plasma was obtained from blood samples after centrifugation (500 × *g* for 10 min at 4°C) and then frozen and stored at −20°C until analysis. The liver was dissected out immediately, weighed, quickly frozen in liquid nitrogen, and stored at −80°C until used.

Table 1
Composition of diet

Ingredient	Content (%)		Ingredient	Content (%)	
	Normal diet	High-fat diet		Normal diet	High-fat diet
Cornmeal*	48.42	31.24	$CaCO_3$	1.3	1.3
Soybean meal†	24.30	29.65	lysine	0.12	0.12
Wheat flour	15.0	7.48	methionine	0.30	0.30
Lard‡	2.2	19.64	choline	0.10	0.10
Cholesterol	0	0.5	minerals	0.04	0.04
Corn bran	7.0	8.41	NaCL	0.20	0.20
$CaHPO_4$	1.0	1.0	vitamins	0.02	0.02

* Cornmeal contains 9.2% protein, 73.8% carbohydrate, and 3.5% fat.
† Soybean meal contains 41.5% protein, 35% carbohydrate, and 5% fat.
‡ Lard provides the following (g/100 g lard): 14:0, 2.0; 14:1, 0.3; 15:1, 0.1; 16:0, 26.5; 16:1, 3.7; 17:0, 0.5; 17:1, 0.4; 18:0, 12.1; 18:1, 42.5; 18:2(ω-6), 9.8; 18:3(ω-3), 0.7; 20:0, 0.2; 20:1, 0.6; 20:4(ω-6), 0.3.

Nutritional and biochemical analyses

For determination of plasma total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triacylglycerol concentrations, the corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, PR China) were used according to the instructions of the manufacturer. The lipoproteins LDL-C and HDL-C were fractionated by a dual-precipitation technique [14]. After fractional precipitation, lipoprotein cholesterol was estimated. Plasma malondialdehyde (MDA) [15], plasma superoxide dismutase (SOD) [16], and total antioxidant capacity (TAC) [17] were also assayed. The weighed frozen liver tissue was homogenized in a glass-Teflon homogenizer with 50 mM phosphate buffer (pH 7.4) to obtain 1:9 (w/v) whole homogenate. The homogenates were then centrifuged at 11 000 × *g* for 15 min at 4°C to discard any cell debris and the supernatant was used for the measurement of MDA, SOD, and TAC. Total protein contents were determined by the method of Lowry et al. [18], using bovine serum albumin as a standard.

DNA microarray procedure

The RNA was prepared from liver of four mice per group using Trizol (P/N 15596-018; Invitrogen Life Technologies, Carlsbad, CA, USA) and subsequently pooled per group. Pooled RNA was further purified using Qiagen RNeasy (P/N 74104; RNeasy Mini Kit, Qiagen) columns and the quality verified by the laboratory on a chip analysis (Bioanalyzer 2100; Agilent, Amstelveen, The Netherlands). Ten micrograms of RNA was used for one-cycle cRNA synthesis (Affymetrix, Santa Clara, CA, USA). Hybridization, washing, and scanning of Affymetrix GeneChip mouse genome 430A arrays was done according to standard Affymetrix protocols.

DNA microarray data analysis

The GeneArray TM scanner 3000 (Affymetrix) was used to scan and quantitatively analyze the scanned image, and the data were globally scaled to all the probe sets with an identical target intensity value. Once the probe array had been scanned, GeneChip software automatically calculated intensity values for each probe cell and made a presence or absence call for each mRNA. Algorithms in the software used probe cell intensities to calculate an average intensity for each set of probe pairs representing a gene that directly correlated with the amount of mRNA. The statistical algorithms were implemented in Affymetrix Microarray Suite 5.0. Expression patterns for each group (HFD and HFD +