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Sesame oil mitigates nutritional steatohepatitis via attenuation of oxidative stress and inflammation: a tale of two-hit hypothesis☆,☆☆

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Abstract

Nonalcoholic fatty liver disease, the most common chronic liver disorder worldwide, comprises conditions from steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. NASH is associated with an increased risk of hepatocellular carcinoma. Sesame oil, a healthful food, increases resistance to oxidative stress, inflammation and protects against multiple organ injury in various animal models. We investigated the protective effect of sesame oil against nutritional steatohepatitis in mice. C57BL/6 J mice were fed with methionine-choline deficient (MCD) diet for 28 days to induce NASH. Sesame oil (1 and 2 ml/kg) was treated from 22nd to 28th day. Body weight, steatosis, triglycerides, aspartate transaminase, alanine transaminase, nitric oxide, malondialdehyde, tumor necrosis factor-α, interlukin-6, interleukin-1β, leptin, and transforming growth factor-β1 (TGF-β1) were assessed after 28 days. All tested parameters were higher in MCD-fed mice than in normal control mice. Mice fed with MCD diet for 4 weeks showed severe liver injury with steatosis, oxidative stress, and necrotic inflammation. In sesame-oil-treated mice, all tested parameters were significantly attenuated compared with MCD-alone mice. Sesame oil inhibited oxidative stress, inflammatory cytokines, leptin, and TGF-β1 in MCD-fed mice. In addition, histological analysis showed that sesame oil provided significant protection against fibrotic collagen. We conclude that sesame oil protects against steatohepatitic fibrosis by decreasing oxidative stress, inflammatory cytokines, leptin and TGF-β1.

Keywords: Sesame oil; Non-alcoholic steatohepatitis; Hepatic fibrosis; Inflammatory cytokines; Leptin; Oxidative stress

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of hepatic abnormalities extends from isolated steatosis (triglyceride accumulation) to steatohepatitis [non-alcoholic steatohepatitis (NASH)], and steatofibrosis. NASH is the progressive form of the disease that can lead to fibrosis, cirrhosis and hepatocellular carcinoma [1,2]. NAFLD is one of the three main causes of cirrhosis [2,3] and increases the risk of liver-related death and hepatocellular carcinoma [4]. Progression of NAFLD to NASH has been modeled by a "2-hit" hypothesis [5]. The first hit involves excess hepatic triglyceride and cholesterol accumulation [6]. The first hit sensitizes the liver to the second hit, which consists of elevated hepatic oxidative stress, inflammation, and insulin resistance. NASH is characterized by increased steatosis, triglycerides, hepatic injury, oxidative stress, inflammatory cytokines, leptin and TGF-β1 [7–10]. Although management of lifestyle (diet and exercise) is one approach to control the onset and progression of NAFLD, the best strategy for managing NAFLD has yet to be defined [11].

Sesame oil, regarded as a superior vegetable oil and ranks second after olive oil in nutritional value [12]. It shows a remarkable stability to oxidation. This could be attributed to endogenous antioxidants (sesamol, sesamolin and sesamin) together with tocopherol [12,13].

Sesame oil is a source of vitamin E and B6. It also contains micronutrients, magnesium, copper, calcium, iron and zinc. Its biological action includes decreased blood pressure, hyperlipidemia and lipid peroxidation by increasing enzymatic and nonenzymatic antioxidants [14]. However, neither the effective treatment of NASH nor the anti-NASH action of sesame oil has been established. Therefore, we hypothesized that sesame oil would attenuate NASH, and we tested the effects of sesame oil on NASH in mice.

2. Materials and methods

2.1. Animals

Male C57BL/6 J mice 7–8 weeks old and weighing 25–30 g was purchased from our institution's Laboratory Animal Center. They were given pellet feed methionine and

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choline deficient (MCD) diet and water ad libitum. They had a 12-h light/dark cycle and central air conditioning (25°C, 70% humidity) throughout the experiment. The animal care and experimental protocols were in accordance with nationally approved guidelines (No. 99054).

2.2. Diet and chemicals

MCD diet and methionine and choline sufficient (MCS) diet were purchased from Test Diet (A Purina Mills, LLC/PMI Nutrition International Company). All the chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Model

Methionine and choline-deficient (MCD) diet-treated mice were used as an experimental model of NASH. The approach has been reported to be a model of NASH [15].

2.4. Experiment I: animal model

The mice were divided into 2 groups (n=6). Group I and II, mice were fed with MCS and MCD diets, respectively. The mice were killed on 22nd day. Serum were collected for alanine transaminase (ALT) and aspartate transaminase (AST); pieces of liver tissue were harvested for hematoxylin and eosin (H&E) stain, steatosis, triglycerides, nitric oxide, malondialdehyde, peroxisome proliferator-activated receptor (PPAR)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-6.

2.5. Experiment II: sesame oil treatment protocol

The mice were divided into 4 groups (n=6). Group I, mice were fed with MCS diet for 28 days. Groups II-IV mice were fed with MCD diet to induce steatohepatitis. Seven doses of mineral oil (2 ml/kg per day) were given orally from 22nd to 28th days in Group I. Seven doses of mineral oil (2 ml/kg per day) were given orally from 22nd to 28th days in Group II. Seven doses of sesame oil (1 or 2 ml/kg per day) were given orally from 22nd to 28th days in Groups III and IV. The body weights were measured every 3 days. The mice were killed on 29th day.

Serum were collected for ALT and AST and pieces of liver tissue were harvested for H&E stain, Sirius red stain, steatosis, triglycerides, nitric oxide, malondialdehyde, TNF-α, IL-6, IL-1β, leptin, and TGF-β1.

2.6. Blood collection

Blood samples were collected from the interior vena cava under light ethyl ether anesthesia.

Blood was drawn by venipuncture into serum separation tube, allowed to clot for 20–30 min at room temperature, and then centrifuged at 15000 rpm at 4°C for 15 min.

2.7. Steatosis assessment

Pieces of liver tissue from mice were cut and placed in 10% formalin. The tissues were dehydrated using a graded percentage of ethanol and then fixed in paraffin wax for 1 h to form blocks. The blocks were trimmed and cut into 4 μm thick section, stained with H&E, and then mounted sections using Depex-Polystyrene dissolved in xylene mountant.

The sections of liver tissue were examined under microscope (magnification ×100) to assess hepatic steatosis. To quantify the degree of steatosis, we used the modified scoring system [16].

The intensity of total steatosis was classified from 0 to 4 (0: <5% of tissue affected by steatosis; 1: 5% to 25% of tissue affected by steatosis; 2: 25 to 50% of tissue affected; 3: 50 to 75% of tissue affected; and 4: more than 75% of tissue affected).

2.8. Measuring nitrite content

Briefly, the amount of nitrite in liver tissue was measured after the Griess reaction. Liver tissue was homogenized in deionized water (1:10, wt/vol). Tissue homogenate (500 μl) was centrifuged at 2500×g for 10 min at 4°C. Supernatant (100 μl) was incubated with 100 μl of Griess reagent at room temperature for 20 min. The absorbance was measured at 550 nm using the spectrophotometer. Nitrite concentration was calculated by comparing it with a standard solution of known sodium nitrite concentration [17].

2.9. Measuring lipid peroxidation levels

Liver tissue was homogenized in Tris–HCl (20 mmol/L; pH 7.4). Tissue homogenate (500 μl) was centrifuged at 2500×g for 10 min at 4°C, and the supernatant (200 μl) was measured for lipid peroxidation (Lipid Peroxidase Assay Kit; Calbiochem-Novabiochem, Darmstadt, Germany) using the spectrophotometer read at 586 nm.

Table 1
Animal modeling of MCD diet induced NASH

Parameter	MCS	MCD
Body weight (g)	23±1.4	17±0.8*
Steatosis (grade)	0.5±0.5	2.8±0.4*
TG (μg/g liver)	9.3±1.5	36.5±4.9*
PPAR-α (pg/mg protein)	113.3±23.4	53.7±3.9*
ALT (U/l)	28.67±7.77	141.67±3.21*
AST (U/l)	52.33±8.62	123.33±4.51*
NO (nmol/mg protein)	0.76±0.06	2.50±0.16*
MDA (pmol/mg protein)	53±10	160±20*
TNF-α (pg/mg protein)	70.42±5.04	111.56±13.69*
IL-6 (pg/mg protein)	38.85±6.45	73.15±7.75*

Data are means±SD.
* MCS vs. MCD significant at P<.05.

2.9.1. Measuring TNF-α, IL-1β, IL-6, leptin, and TGF-β1 levels in liver tissue

Liver tissue was homogenized in deionized water (1:10; wt/vol) and centrifuged at 12500×g for 10 min at 4°C. The TNF-α, IL-1β, IL-6, leptin and TGF-β1 levels in the tissue supernatant were determined using an enzyme-linked immunosorbent assay (R&D Systems, Inc, Minneapolis, MN, USA).

TNF-α, IL-1β, IL-6, leptin and TGF-β1 were assessed by measuring absorbance at 450 nm and extrapolating from a standard curve with a sensitivity limit of 32.5 pg/ml. Protein concentration (pg/mg) in liver tissue was determined using protein assay dye (Bio-Rad Laboratories, Hercules, CA, USA).

2.9.2. Fibrosis assessment

Pieces of liver tissue were cut and placed in 10% formalin. The tissue pieces were dehydrated using a graded percentage of ethanol and then fixed in paraffin wax for 1 h to form blocks. The blocks were trimmed and cut into 4-μm-thick sections. The sections were de-waxed and hydrated and stained with Weigert's haematoxylin for 8 min, then washed the slide for 10 min in running tap water. The slides were stained with Sirius red (Sigma) for 1 h and washed in two changes of acidified water. The slides were then dehydrated in 100% ethanol, and cleared in xylene. The sections of liver tissue were examined under microscope (original magnification ×100) to assess fibrosis grade. To quantify the fibrosis grade, we used the modified scoring system [18]. The grade of hepatic fibrosis was classified from 0 to 4 (0: no fibrosis; 1: portal fibrosis without septa; 2: portal fibrosis with few septa; 3: numerous septa without cirrhosis; and 4: cirrhosis).

2.9.3. Statistical analysis

All statistical analyses were done using SPSS 11.0.1 (SPSS Inc., Chicago, IL). Data are means±S.D. Differences in the measured variables between each group were assessed using Fisher's least significant difference test. Significance was set at P < .05.

3. Results

3.1. MCD diet induced NASH in mice

MCD-fed mice showed significant increase in steatosis, triglycerides, ALT, AST, nitric oxide, malondialdehyde, TNF-α, and IL-6

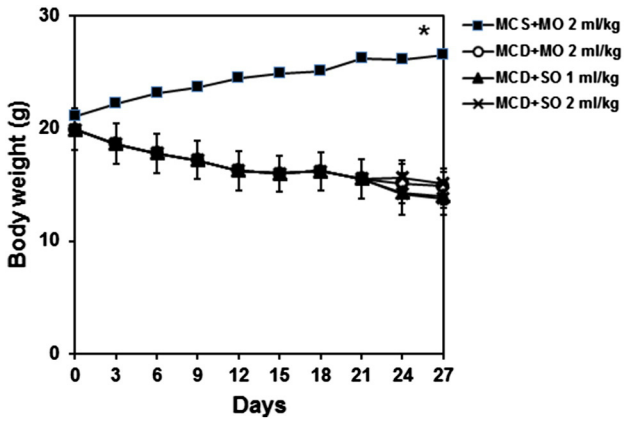

Fig. 1. Effect of sesame oil on body weight in MCD-fed NASH mice. Group I mice (n=6) were fed with MCS diet and gavaged with mineral oil from day 22–28; Group II mice (n=6) were fed with MCD diet and gavaged with mineral oil from day 22-28; Group III and IV mice (n=6 each) were fed with MCD diet and gavaged with oral sesame oil (1 and 2 ml/kg, respectively) from day 22–28.