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Daily sesame oil supplement attenuates joint pain by inhibiting muscular oxidative stress in osteoarthritis rat model

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Abstract

Osteoarthritis (OA) is the most common form of arthritis, affecting approximately 15% of the population. The aim of this study was to evaluate the efficacy of sesame oil in controlling OA pain in rats. Rat joint pain was induced by medial meniscal transection in Sprague–Dawley rats and assessed by using hindlimb weight distribution method. Muscular oxidative stress was assessed by determining lipid peroxidation, reactive oxygen species and circulating antioxidants. Sesame oil significantly decreased joint pain compared with positive control group in a dose-dependent manner. Sesame oil decreased lipid peroxidation in muscle but not in serum. Further, sesame oil significantly decreased muscular superoxide anion and peroxynitrite generations but increased muscular glutathione and glutathione peroxidase levels. Further, sesame oil significantly increased nuclear factor erythroid-2-related factor (Nrf2) expression compared with positive control group. We concluded that daily sesame oil supplement may attenuate early joint pain by inhibiting Nrf2-associated muscular oxidative stress in OA rat model.

Keywords: Osteoarthritis; Joint pain; Oxidative stress; Sesame oil; Rats

1. Introduction

Osteoarthritis (OA) is the most common form of arthritis, affecting approximately 15% of the population [1]. OA is the leading cause of lower extremity disability among older adults, with the estimated lifetime risk for knee OA being approximately 40% in men and 47% in women [2]. By 2030, it is predicted that 67 million people in the United States will be diagnosed with OA [3]. Therefore, OA is now considered as a major public health problem worldwide [4].

Because there is no current pharmacologic agent to prevent OA progression, the goal of pharmaceutical therapy is to control pain. Acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for managing OA pain. However, gastrointestinal and cardiovascular adverse reactions are frequently observed in patients with long-term treatment of NSAIDs [5]. Long-term acetaminophen may cause hepatic dysfunction and injury [6]. It would be important to develop a new approach for managing patients with OA pain.

Quadriceps muscle weakness is one of the important factors involved in the pathogenesis of OA [7]. Although muscle weakness has been considered as a secondary effect in knee OA historically, recent studies suggest that quadriceps muscle weakness may precede the onset of radiographic evidence of OA and pain [8]. Initiating OA in the guinea pig is associated with the changes in the quadriceps skeletal muscle [9]. Quadriceps muscle weakness may be directly involved in the pathogenesis and development of OA and pain [10].

Oxidative stresses are believed to be involved in the development and progression of OA. Oxidative stress is imposed on cells as a result of one of two factors: an increase in oxidant generation and a decrease in antioxidant protection. If oxidant attacks continuously, oxidation of lipid constituents of membranes ensue, which impairs the function of cell organelles and eventually culminates in ultrastructural injury [11]. Reactive oxygen species (ROS), such as superoxide anion and peroxynitrite, are produced in animals and humans under pathophysiologic conditions [12,13]. Superoxide anion can act with nitric oxide to generate peroxynitrite [14]; both of them are the important mediators of lipid peroxidation (LPO).

Glutathione peroxidase (GPx), localized in the cytosol and the inner membrane of mitochondria of animal cells, is a crucial enzyme in the biosynthesis of glutathione (GSH). GSH, as a potent free radicals scavenger, plays an important role in detoxification and cellular defense [15–17]. It prevents interactions of reactive intermediates with critical cellular constituents, such as phospholipids of biomembranes, nucleic acids and proteins [15–17]. In addition, nuclear factor erythroid-2-related factor (Nrf2), a basic leucine zipper protein transcription factor, initiates transcription of antioxidative genes and their proteins, such as GPx [18].

Sesame oil, derived from the plant species *Sesamum indicum* L., consists of fatty acids and lignans. The fatty acids are palmitic acid

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(16:0; 7.0%–12.0%), palmitoleic acid (16:1; less than 0.5%), stearic acid (18:0; 3.5%–6.0%), oleic acid (18:1; 35%–50%), linoleic acid (18:2; 35%–50%), linolenic acid (18:3; less than 1%) and eicosenoic acid (20:1; less than 1%). The nonfat portion (1%–2%) contains lignans such as sesamin, sesamol, sesamolin, sesaminol and episesamin. Recently, sesame oil has been suggested to have excellent antioxidative property in various disease models [19–21]. It is very possible that sesame oil can prevent the OA development by its potent antioxidative property; however, the effect of sesame oil on OA development has never been investigated. The aim of the study was to examine the preventive effect of sesame oil on medial meniscal transection (MNX)-induced OA in rats.

2. Materials and methods

2.1. Materials

Sesame oil was purchased from Sigma (St. Louis, MO, USA).

2.2. Animals

Male SPF Sprague–Dawley rats weighing 200–300 g were obtained from our institution's Laboratory Animal Center. They were individually housed in a room with a 12-h dark/light cycle and central air conditioning (25°C, 70% humidity), allowed free access to tap water and fed a rodent diet from Richmond Standard, PMI Feeds, Inc. (St Louis, MO, USA), with or without a sesame oil supplement. The animal care and experimental protocols were in accordance with nationally approved guidelines.

2.3. OA induction in rats

Rat OA was induced by MNX surgery. It was performed under 3.5% isofluorane inhalational anesthetics. Rats received cephalexin (Ceporex oral drops) (0.03 ml/100 g body weight) 1 h before and 12, 24 and 36 h after surgery. A small incision was made longitudinally down the medial side of the knee, and a cauterizer was used to work through both the connective tissue and muscle layers until the medial collateral ligament, anchoring the medial meniscus to the tibial plateau, was identified. The ligament was grasped at the tibial end and cut until fully transected. The ligament was then transected again at the femoral end to remove the portion overlying the meniscus. The meniscus was freed from the fine connective tissue, allowing a full-thickness MNX. Sham animals underwent the same surgical procedure with the omission of MNX [22].

2.4. Experimental designs

2.4.1. Experiment I

A time-course study of OA-associated joint pain in rats was performed. Rats were divided into two groups (n=5): sham group (rats that received sham operation) and OA group (rats that received MNX surgery). The levels of OA pain were assessed 0, 1, 4, 7 and 14 days after sham or MNX operation.

2.4.2. Experiment II

Effects of sesame oil on OA-associated joint pain in rats were studied. Rats were divided into five groups (n=5): sham group (rats that received shame operation), OA group (rats that received MNX surgery only) and SO1, SO2 and SO4 groups [rats that ingested 1, 2 or 4 ml/kg of sesame oil (by using a feeding tube) daily for 7 days, respectively, after MNX]. The levels of OA pain were assessed 0, 1, 4 and 7 days after sham or MNX operation. Rat quadriceps muscle near the area of sham or MNX operation was collected at 7 days after operation. Muscular LPO, GSH, superoxide anion, peroxynitrite and GPx expression as well as nuclear Nrf2 expression levels were determined.

2.5. Assessing joint pain in rats

OA-associated joint pain was assessed by using an incapacitance meter (IITC, Inc., Woodland Hills, CA, USA), a behavioral analysis assay that measures weight bearing on the hindlimbs while the animal was in an induced rearing posture. In brief, an incapacitance meter consists of two scales and specialized caging to encourage a rearing posture. Weight on the left and right hindlimbs was acquired during 5-s intervals (five trials per rat). These data were converted into weight distribution by dividing the weight on the right limb by the total weight for both hindlimbs. Weight distribution imbalance was determined at each time point by using a repeated-measures test, with balanced weight distribution represented by a right limb percentage weight near 50% [23].

2.6. Measuring serum and muscular LPO levels

Muscle tissues were homogenized in Tris–HCl (20 mM, pH 7.4). Whole blood (500 μl) or muscular tissue homogenate was centrifuged at 2500×g for 10 min at 4°C. Supernatant (200 μl) was taken for LPO measurement by using a commercial assay kit (Lipid Peroxidase Assay Kit; Calbiochem-Novabiochem Co., Darmstadt, Germany) following the manufacturer's instruction and a spectrophotometer reading at 586 nm. The protein concentration in tissue homogenate was determined by using protein assay dye (Bio-Rad Laboratories, Hercules, CA, USA). Bovine serum albumin (BSA) (0, 2, 4, 6, 8 and 10 mg in 1 ml) was used for establishing standard curve. Briefly, 1 μl of sample or BSA was added into 1 ml of diluted assay dye (1:5 v/v), and the absorbance at 595 nm was measured immediately.

2.7. Measuring muscular GSH levels

Muscle tissues were homogenized in ice-cold trichloroacetic acid (0.1 g of tissue plus 1 ml of 10% trichloroacetic acid). Briefly, after the homogenates had been centrifuged at 3000 rpm for 10 min, 500 μl of supernatant was added to 2 ml of 0.3 M Na2HPO4 solution. A 200-μl solution of dithiobisnitrobenzoate (in 1% sodium citrate, 0.4 mg/ml) was added, and the absorbance at 412 nm was measured immediately [13].

2.8. Determining muscular superoxide and peroxynitrite levels

Briefly, tissue was homogenized in Tris–sucrose buffer (0.24 M sucrose in 20 mM Tris–HCl buffer containing 1 mM EDTA (pH 7.4) (1:10; wt/vol)]. The homogenate was centrifuged at 400×g at 4°C for 30 min. Superoxide anion and peroxynitrite were measured using a high-performance chemiluminescence (CL) analyzer (CLA-2100; Tohoku Electronic Industrial Co., Ltd, Rifu, Japan). Briefly, 400 μl of tissue homogenate was mixed with 200 μl of phosphate buffer solution in a stainless dish, and then the background CL count was read for 60 s. One hundred microliters of lucigenin or luminol (17 mM dissolved in phosphate buffer solution to determine superoxide anion or peroxynitrite, respectively) was injected into the machine, and the CL was counted for another 1200 s at 10-s intervals. The data were analyzed using Chemiluminescence Analyzer Data Acquisition Software (Tohoku Electronic Industrial Co.) [13].

2.9. Western blotting

Nuclear extraction kit (Sigma) was used to separate nuclear and cytosolic protein. Fifty micrograms of protein was loaded on sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to nitrocellulose sheets (NEN Life Science Products, Inc., Boston, MA, USA). After blocking, the blots were incubated with Nrf2 (Novus Biologicals, Littleton, CO, USA), GPx (R&D Systems, Minneapolis, MN, USA) or β-actin antibody (Merck Millipore, Darmstadt, Germany) (dilution 1:1000) in 5% nonfat skim milk (using β-actin as a loading control). After washing, the blots were incubated with secondary antibodies conjugated with alkaline phosphatase (dilution 1:3000) (Jackson ImmunoResearch Laboratories, Inc., Philadelphia, PA, USA). Immunoblots were developed using bromochloroindolyl phosphate/nitroblue tetrazolium solution (Kirkegaard and Perry Laboratories, Inc., Baltimore, MD, USA).

2.10. Statistical analysis

Data were expressed as the means±standard deviation (SD). One-way analysis of variance followed by Student's t test analysis was used to make pairwise comparisons between the groups. Statistical significance was set at P<.05.

3. Results

3.1. Effect of sesame oil on OA-associated joint pain in rats

To examine the effect of sesame oil on OA development, OA-associated joint pain in rats was assessed. A time-course study of OA-associated joint pain in rats was performed. Pain levels were significantly increased in OA group at 1, 4, 7, and 14 days afetr MNX surgery compared with that in Sham group (Fig. 1). Sesame oil at the dose of 1 ml/kg significantly increased the weight distribution compared with OA group at 1 day after OA induction. Further, the percentage of weight distribution is significantly higher in SO1, SO2 and SO3 groups compared with that in sham groups at 4 and 7 days after OA induction by MNX (Fig. 2).