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Dietary perilla oil lowers serum lipids and ovalbumin-specific IgG1, but increases total IgE levels in ovalbumin-challenged mice

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ABSTRACT

Our previous studies indicated that α-linolenic acid (ALA)-rich perilla oil might alleviate bronchoalveolar inflammation. However, it failed to modulate the Th1/Th2 balance toward the Th1 pole during Th2-skewed allergic airway inflammation in mice. This study attempts to further investigate the effects of dietary perilla oil on serum lipids and immunoglobulin profiles using an ovalbumin (OVA)-challenged mouse model. The inbred female BALB/c mice were randomly divided into four groups and fed different AIN-76 feeds containing 5% corn oil (rich in linoleic acid, 18:2n-6 polyunsaturated fatty acids (PUFA), as a control diet), 5% perilla oil (rich in α-linolenic acid, 18:3n-3 PUFA) or 5% compound oil containing 50% corn oil and 50% perilla oil, respectively, for 35 consecutive days *ad libitum*. Experimental mice were sensitized by an intraperitoneal injection of alum-precipitated antigen containing ovalbumin on 7, 14 and 21 days after supply of the specified experimental diets. One week later, the mice were then challenged by aerosolized OVA. The results showed that dietary perilla oil administration significantly ($P < 0.05$) decreased the relative liver tissue weight (RTW) and serum lipid levels including triglycerides, total cholesterol, HDL- and LDL-cholesterol. However, the HDL/LDL ratio was also significantly lowered by dietary perilla oil. Dietary perilla oil markedly decreased serum OVA-specific IgG1 level and total IgA antibodies (Th2 antibodies). Unfortunately, it also increased non-specific serum IgE (Th2 antibody) levels. The results suggest that dietary perilla oil might have a moderately beneficial effect on asthmatic allergy via lowering serum lipids and OVA-specific IgG1, as well as total IgA levels. However, it failed to obviously modulate Th1/Th2 antibody levels via isotype switching of B cells from Th2 antibody to Th1 antibody.

1. Introduction

Changes in dietary lipid pattern, such as imbalance in the n-6/n-3 polyunsaturated fatty acids (PUFAs), may be promoters of many chronic diseases including atherosclerosis, essential hypertension, obesity, diabetes and many cancers due to the increased production of pro-inflammatory cytokines and eicosanoids *in vivo* (Simopoulos, 1991, 2002a). Different kinds of dietary fat with varying fatty acid compositions have been judged to influence plasma and tissue lipid appearance (Yamamoto et al., 1987; Surette et al., 2004). The positive or negative impacts on serum lipid profiles by dietary fats are associated with human health (Wahrburg, 2004; Connor, 2000). The effects of n-6/n-3 fatty acid ratios in dietary oil on health have been much discussed.

Long-chain n-3 (PUFAs) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from oily fish and fish oil are reported to have beneficial biological effects in improving human health (Simopoulos, 2002b; Sijben and Calder, 2007). Dietary n-3 PUFAs reduces the risk for coronary heart disease by lowering the heart rate and blood pressure, decreasing platelet aggregation and lowering triglyceride levels (Harris et al., 2008). Both EPA and DHA can attenuate T-cell-mediated inflammation (Switzer et al., 2004), decrease the production of arachidonic acid-derived mediators and pro-inflammatory cytokines, and modulate adhesion molecule expression (Calder, 2005).

Perilla frutescenes is an edible plant frequently used as one of the most popular garnishes and food colorants in some Asian countries such as China and Japan. Perilla leaf extract has been reported to possess several anti-inflammatory effects, such as inhibition of tumor necrosis factor-α production in male ICR mice (Ueda et al., 2002) and inhibition of seasonal allergic rhinoconjunctivitis in humans (Takano et al., 2004). Perilla seeds are widely used to treat pulmonary diseases in traditional Chinese medicine. Perilla oil from *P. frutescenes* seeds is rich in α-linolenic acid (ALNA, 18:3n-3). ALNA is a plant-derived n-3 fatty acid, which exists in green leafy vegetables, as well as some seed and vegetable oils, is a

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precursor of EPA and DHA (Burdge and Calder, 2005). ALNA-rich perilla oil exerts a positive effect on preventing atherosclerosis and chemically induced cancer, and improves immune and mental function in animal studies (Kurowska et al., 2003). Kim and Choi (2001) indicated that feeding perilla oil diets decreased postprandial plasma lipids in male Sprague-Dawley rats. Dietary perilla oil also exhibits a similar physiological activity to fish oil in modulating hepatic fatty acid oxidation (Ide et al., 2000; Kim et al., 2004). We have also shown that dietary perilla oil inhibits pro-inflammatory cytokine production in the bronchoalveolar lavage fluid of ovalbumin-challenged mice (Chang et al., 2008). However, more evidence concerning the physiological function of dietary perilla oil should be identified, especially under the stress of different diseases such as asthma.

A clinical study has indicated that hypercholesterolemia is a potential risk factor for asthma (Al-Shawwa et al., 2006). Yeh and Huang (2004) reported that allergic pulmonary inflammation is significantly correlated with serum cholesterol levels in male C57BL6 mice sensitized with ovalbumin. Surprisingly, simvastatin, widely used in clinical practice as cholesterol-lowering agents, is effective in reducing BAL total cellularity and eosinophilia in a murine model of allergic asthma (McKay et al., 2004). Recent human and animal studies suggest that hypercholesterolemia is associated with high levels of inflammation-sensitive plasma proteins (Engström et al., 2002), as well as increased expression of pro-inflammatory genes, cellular adhesion molecules, and pro-inflammatory cytokines (Stokes et al., 2002; Scalia et al., 1998). Thus, serum lipid profiles may be related to immunological responses during inflammation.

To understand the effects of dietary perilla oil on the allergic asthma, we have established an asthmatic mouse model to evaluate the anti-inflammatory and immuno-modulatory potential of ALNA-rich perilla oil (Chang et al., 2008; Lin et al., 2006a; Nagai et al., 2004). The female BALB/c mice were divided into four groups and fed different AIN-76 feeds containing 5% corn oil (rich in linoleic acid, 18:2n-6 polyunsaturated fatty acids (PUFA), as a control diet), 5% perilla oil (rich in α-linolenic acid, 18:3n-3 PUFA) or 5% compound oil containing 50% corn oil and 50% perilla oil, respectively, for 35 consecutive days *ad libitum*. Experimental mice were sensitized by an intraperitonal injection (i.p.) of alum-precipitated antigen containing ovalbumin on 7, 14 and 21 days after supply of the specified experimental diets. One week later, the mice were then challenged by aerosolized OVA. We found that perilla oil administration might alleviate bronchoalveolar inflammation by decreasing the secretion of pro-inflammatory cytokines and T-helper type 1 (Th1) cytokines into the local lung and airway tissues compared to corn oil (rich in linoleic acid, LA, 18:2n-6). However, asthma is recognized as a Th2-skewed allergic disease (Kay, 2003). Th2-type cells are important in humoral immunity and defend against extracellular pathogens (Opal and DePalo, 2000) via eosinophilic infiltration, as well as IgE and IgG1 production *in vivo* (Morokata et al., 1999). In contrast to the Th2 cells, Th1 cells promote cell-mediated immunity to destroy intracellular pathogens (Opal and DePalo, 2000), to inhibit eosinophilic infiltration, IgE and IgG1 production, but strengthen IgG2a production *in vivo* (Morokata et al., 1999). Th1-skewed immune responses are generally pro-inflammatory and may result in autoimmune and chronic inflammatory diseases, while Th2-skewed immune responses may cause asthma and other allergies (Kidd, 2003). Although the Th1/Th2 paradigm is well known, there is little discussion on the regulation of immunoglobulin and serum lipid profiles by ALNA-rich perilla oil in an allergic airway inflammation murine model. This study investigates the effects of dietary perilla oil on serum lipids and antibody levels using an ovalbumin (OVA)-sensitized and challenged allergic airway inflammation murine model.

2. Methods

2.1. Materials, experimental animals and feeds

The feed formula was same as that described by Chang et al. (2008). The experimental feed was prepared according to the recommendation of the American Institute of Nutrition AIN-76 (AIN, 1977) that satisfies the nutritional requirement for mouse growth, and varied only in lipid composition. We just replaced the oil component (5%) in AIN-76 feed with the selected experimental oils (5%). The diets are nutritionally balanced. The basic composition of each feed, expressed in g/100 g, contained 40 g sucrose, 25 g corn starch, 20 g casein, 5 g fiber, 3.5 g mineral mixture, 1 g vitamin mixture, 0.3 g DL-methionine, 0.2 g choiline bitartrate and 5 g lipid. The components of each feed were thoroughly mixed and stored at −20 °C. There were three kinds of lipid used in the experiment. Perilla oil (PER) rich in α-linolenic acid (>60.5%) was purchased from MasterAsia Marketing Co. (Taipei, Taiwan, ROC) as the source of n-3 fatty acid. In addition, there are 14.7% linoleic acid, 16.1% oleic acid, 2.0% stearic acid and 6.2% palmitic acid in perilla oil according to the manufacturer's profiles. Corn oil (COR), rich in linoleic acid but poor in α-linolenic acid (<2%), was purchased from Chang Chi Foodstuff Factory Co. (Taichung, Taiwan, ROC) as a control and the source of n-6 fatty acid. The fatty acid composition in corn oil consists of 49.3% linoleic acid, 28.5% oleic acid, 2.1% stearic acid and 12.9% palmitic acid according to the data of Rafalowski et al. (2008). The ratios of n-6/n-3 fatty acids are 0.27 in perilla oil and 61.6 in corn oil, respectively. Compound oil (COR-PER) was a mixture containing 50% corn oil and 50% perilla oil.

The animal use protocol listed below has been reviewed and approved by the Institutional Animal care and Use Committee (IACUC), National Chung Hsing University, Taiwan, ROC. The female BALB/c ByJNarl mice (6 weeks old) were obtained from the National Laboratory Animal Center, National Applied Research Laboratories, National Science Council in Taipei, ROC and maintained in the Department of Food Science and Biotechnology at National Chung Hsing University College of Agriculture and Natural Resources in Taichung, Taiwan, ROC. The animal room was kept on a 12-h light and 12-h dark cycle. Constant temperature (25 ± 2 °C) and humidity were maintained. The mice were housed and kept on a chow diet (laboratory standard diet) to acclimatize for 2 weeks before feeding the experimental diet. After this equilibrium period, mice were randomly divided into four groups (n = 12) varied by oil source and sensitized treatments: non-sensitized control (PBS/COR), dietary control (OVA/COR), OVA/COR-PER and OVA/PER. The initial average body weight of each group showed no significant differences among groups. Each group was fed with the specified experimental diet for 35 consecutive days *ad libitum*. Mice food intake and body weight were measured twice a week during the study period.

2.2. OVA-sensitized and -challenged allergic inflammation murine model

The inflammation murine model was used in our previous study (Chang et al., 2008). The mice (8 weeks old) were sensitized and challenged to induce allergic airway inflammation. The mouse allergic airway inflammation model was manipulated as described by Lin et al. (2006a) and slightly modified at aerosolized-OVA concentration and inhalation time to enhance the induction of airway inflammation. In brief, mice were sensitized by an intraperitonal injection (i.p.) of 0.2 ml alum-precipitated antigen containing 8 μg of ovalbumin (OVA, albumin chicken egg grade III, Sigma, MO, USA) and 2 mg $Al(OH)_3$ to induce primary immunity after supply of the specified experimental diets for one week. Two booster injections of this alum-OVA mixture were given 7 and 14 days later, respectively. Non-sensitized control mice received alum-phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2–7.4, 0.2 μm filtered) only. One week later, the mice were then challenged by aerosolized OVA at a concentration of 5 mg OVA per milliliter PBS for 60 min, twice at 3 day intervals and repeated twice in 24 h. The aerosolized OVA were produced using an ultrasonic nebulizer (sw918, Shinmed). Non-sensitized control mice received only PBS. Two days later, the animals were anesthetized with diethyl ether, exsanguinated by retro-orbital venous plexus puncture and immediately euthanized by CO_2 inhalation. The serum was collected and assayed for antibody and lipid contents. The organs were collected and weighted.

2.3. Assay of total triglycerides, cholesterol, HDL- and LDL-cholesterol

The lipid levels in experimental mouse serum were determined using Randox assay kits: triglycerides (TG) assay kit, total cholesterol (TC) assay kit and high density lipoprotein (HDL)-cholesterol assay kit. Serum samples were appropriately diluted. The lipid levels were assayed according to the manufacturer's instructions. The concentration of low density lipoprotein (LDL)-cholesterol was calculated using the following formula: LDL-cholesterol (mg/dl) = total cholesterol − (TG/5) − HDL.

2.4. OVA-specific IgE, IgG1 and IgG2a assay

An ELISA protocol was used to determine the OVA-specific IgE, IgG1 and IgG2a antibody levels in the serum. Aliquots of 200 μl/well OVA (10 μg/ml dissolved in 0.1 M $NaHCO_3$, pH 8.2) were pipetted into the 96-microwell plate (Nunc), respectively. Plates were incubated overnight at 4 °C. The plates were then carefully