HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2017.07.019

A single administration of fish oil inhibits the acute inflammatory response in rats

Laura Lícia Milani de Arruda[1], Franciele Queiroz Ames[1], Damila Rodrigues de Morais[2], Renata Grespan[1], Ana Paula Maziero Gil[1], Maria Angélica Raffaini Covas Pereira Silva[1], Jesuí Vergílio Visentainer[2], Roberto Kenji Nakamura Cuman[1], Ciomar Aparecida Bersani-Amado[1]✉

[1]*Department of Pharmacology and Therapeutics, State University of Maringá, Maringá, PR, Brazil*

[2]*Department of Chemistry, State University of Maringá, Maringá, PR, Brazil*

ARTICLE INFO

Article history:
Received 13 May 2017
Received in revised form 18 Jun 2017
Accepted 16 Jul 2017
Available online 22 Aug 2017

Keywords:
Omega-3 fatty acid
Polyunsaturated fatty acid
Pleurisy
Leukocyte migration
EPA
DHA

ABSTRACT

Objective: To investigate the antiinflammatory effects of a single administration of fish oil (FO) on the acute inflammatory response.
Methods: The paw edema and pleurisy models were used to evaluate the effects of FO dissolved in olive oil (FOP) orally administered in a single dose in rats. Nitric oxide (NO) concentrations in the pleural exudate were performed according to the Griess method and the cytokine concentrations were determined by Luminex bead-based multiplex assay.
Results: FOP treatment (30 and 300 mg/kg) significantly reduced paw edema. FOP treatment at 18.75, 37.5, 75.0, 150.0, and 300 mg/kg decreased both the volume of pleural exudate and cellular migration into the pleural cavity and each of these doses presented the same effectiveness. Treatment with FOP (300 mg/kg) reduced NO, TNF-α, IL-1β, and IL-6 concentrations in the pleural exudate.
Conclusions: The present data provide evidence that FO has inhibitory effects on the acute inflammatory response when administered in a single dose in rats. This effect might be attributable to a direct inhibitory effect of FO on the production or release of inflammatory mediators that are involved in the pathological processes evaluated herein.

1. Introduction

Inflammation involves a complex response of vascularized living tissue to harmful stimuli and is often associated with pain. This process involves such characteristic events as increases in vascular permeability, local blood flow, and the migration of leukocytes in an attempt to eliminate the offending agent and heal tissue lesions. However, if these events are not properly resolved, then inflammation can develop into more serious conditions, with consequent increases in the proliferation of granulomatous tissue and loss of organ function [1,2].

The currently available drugs that are used for the clinical treatment of inflammatory diseases include nonsteroidal antiinflammatory drugs (NSAID), steroidal antiinflammatory drugs, biological agents (*e.g.* tumor necrosis factor inhibitors) and immunosuppressive drugs [3]. Unfortunately, these treatments are often ineffective and require high doses or prolonged periods of treatment, thus causing adverse effects on the gastrointestinal tract, kidneys, and liver [4,5]. Thus, the use of natural products or traditional medicines that have favorable therapeutic effects but fewer adverse effects has gained interest in the treatment of inflammatory diseases [6].

Polyunsaturated fatty acids (PUFA) participate in the formation of membrane phospholipids and are important in the prevention and treatment of inflammatory diseases, autoimmune diseases, coronary diseases, hypertension, and arthritis [7–9]. One group of PUFA has a first double bond that is located on the third or sixth carbon atom from the terminal methyl carbon. These groups are referred to as omega-3 (n-3) and omega-6 (n-6), respectively [7].

The animal body lacks desaturase enzymes, which insert double bonds between carbons 3 and 4 and between carbons 6 and 7. Therefore, they are essential for mammals and must be obtained through the diet [7].

First author: Laura Lícia Milani de Arruda, Department of Pharmacology and Therapeutics, State University of Maringá, Maringá, PR, Brazil.
✉Corresponding author: Ciomar Aparecida Bersani-Amado, Department of Pharmacology and Therapeutics, State University of Maringá, Maringá, PR, Brazil.
Tel: +55 44 30115166
E-mail: cabamado@uem.br
Peer review under responsibility of Hainan Medical University.
Foundation project: This study was financially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

The main representatives of the n-3 family are α-linolenic acid (ALA; 18:3n-3), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3). The main representatives of the n-6 family are linoleic acid (LNA; 18:2n-6) and arachidonic acid (ARA; 20:4n-6) [7]. The fatty acids of both families, n-3 and n-6, are found in vegetable oils, whereas the oil that is extracted from certain cold-water fish (*e.g.* sardines, salmon, anchovies) is the richest source of n-3, mainly EPA and DHA.

Omega-3 PUFA have well-established antiinflammatory properties [10,11]. They have also been proposed to have antioxidant actions, especially DHA [12]. Double-blind studies in patients with rheumatoid arthritis who used diet supplementation with n-3 PUFA for a long period of time reported significant improvements in symptoms, with a reduction of the number of swollen joints, pain relief, and a reduction of traditional antiinflammatory drugs use [11,13,14].

However, to our knowledge, although the antiinflammatory actions of n-3 are well established, few studies have been conducted to evaluate the antiinflammatory activity of n-3 PUFA with acute treatment without supplementation in the diet for an extended period of time. Research on the effectiveness of fish oil (FO) is justified because it is the richest natural source of DHA and EPA, with commercially available standards containing high concentrations of n-3 fatty acid formulations. The present study evaluated the effects of a single administration of FO on the acute inflammatory response.

2. Materials and methods

2.1. Animals

A total of 142 male Wistar rats, weighing 200–220 g (7–8 weeks old), were kept in an environment under controlled temperature (22 ± 2) °C and a 12 h/12 h light/dark cycle with free access to a standard pellet diet and water. The experimental protocol was approved by the Ethics Committee for Animal Experimentation of the State University of Maringa (ECAE/UEM 045/2012).

2.2. Fish oil preparation

The FO that was used in the present study was commercially obtained from Naturalis (São Paulo, Brazil). The OMEGA 3 DHA 250 product (triglycerides form) was chosen because of its high DHA content. According to the manufacturer, each 500 mg capsule contains 250 mg DHA and 50 mg EPA. For the experiments, the contents of the FO capsule were diluted in olive oil (OO).

Initially, the fatty acids that were present in the FO capsule were quantified to validate the aforementioned information from the manufacturer. The composition of the fatty acids that were present in the FO preparations (FOP) and OO as a diluent was also evaluated. In this assay, two FOP that contained FO (10 and 100 mg/mL) diluted in OO were used. For the calculations that were used for the preparations, we considered the amount of DHA that was contained in the FO capsule (250 mg/capsule).

2.3. Chemical evaluation of lipid profile

Fatty acid methyl esters (FAMEs) were prepared by transesterification of the total lipids as described by Maia and Rodriguez-Amaya [15]. The FAMEs were then separated by gas chromatography with a Thermo 3 300 gas chromatograph fitted with a flame ionization detector and fused-silica CP-7420 (SELECT FAME) capillary column (100 m × 0.25 mm internal diameter and 0.25 μm of cyanopropylpolysiloxane). The operation parameters were the following: detector temperature, 230 °C; injection temperature, 220 °C; column temperature, 185 °C for 10 min, programmed to increase at 4 °C/min up to 235 °C, maintained for 5 min; carrier gas, hydrogen (1.2 mL/min; ultrapure; White Martins, Rio de Janeiro, RJ, Brazil); makeup gas, nitrogen (30 mL/min); split injection at a 1:80 ratio. The percentages were determined by integration of the peak areas using CHROMQUEST 5.0 software (Thermo Fisher Scientific, Inc., Waltham, USA). Individual FAME were identified by comparing the retention times of the samples with standards from Sigma (St. Louis, MO, USA) and coelution with standards of known compositions. The FAMEs were expressed as mole% of fatty acids.

2.4. Induction of paw edema

This experiment was performed to determine the amount of FO that effectively inhibited the inflammatory response. For this assay, FOP (30 and 300 mg/kg; n = 7/group, respectively), OO (n = 5), indomethacin (Indo; 5 mg/kg; n = 5), and water (n = 5) were orally administered in a single dose in different groups of rats, which were fasted for 15 h. The treatment was realized 1 h before induction of paw edema.

Paw edema was induced by intradermally injecting 0.1 mL of a suspension of carrageenan (Cg; 200 μg/paw, dissolved in sterile saline) in one of the hind paws of the rats. In the contralateral paw, an equal volume of saline was injected according to Winter *et al* [16]. The paw volume (in microliters) to the tibial-tarsal joint was measured 1, 2, and 4 h after the carrageenan injection using a digital plethysmograph (Ugo Basile). Paw edema was expressed in terms of the increase in paw volume by subtracting the volume of the paw that received saline (control paw) from the volume of the paw that received carrageenan.

2.5. Induction of pleurisy

In the pleurisy model, the rats were treated with different amounts of FOP to determine whether the difference in intensity of the antiinflammatory effect depended on the amount of FOP that was administered. FOP (18.75, 37.5, 75, 150, and 300 mg/kg; n = 8, 8, 6, 6, and 8/group, respectively), pure fish oil (FO; 300 mg/kg; n = 7), OO (n = 7), indomethacin (5 mg/kg; n = 6), and water (n = 8) were administered orally in a single dose in the different groups of rats, which were fasted for 15 h. A normal animals group (n = 6) without receiving pleural injection of carrageenan was also evaluated.

Pleurisy was induced by injecting 0.25 mL of a suspension of carrageenan (200 μg) into the intrapleural cavity 1 h after the respective treatments according to the technique described by Vinegar *et al* [17]. Carrageenan was diluted in phosphate-buffered saline (PBS; pH 7.4). Four hours later, the animals were anesthetized and sacrificed for the collection of pleural inflammatory exudate. The collected material was transferred to centrifuge tubes, and the total volume was determined. After centrifugation at 2 500 rotations per minute (rpm) for 10 min, the