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Participation of the anti-inflammatory and antioxidative activity of docosahexaenoic acid on indomethacin-induced gastric injury model

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ABSTRACT

Adverse gastrointestinal (GI) effects caused by nonsteroidal anti-inflammatory drugs (NSAIDs), including indomethacin, are recognized as the major limitation to their clinical use. NSAID-induced gastric damage is generated by cyclooxygenase inhibition, activation of inflammatory processes, and oxidative stress. Docosahexaenoic acid (DHA), an omega-3 polyunsaturated fatty acid, has shown gastroprotective effects; however, the molecular mechanisms underlying these effects have not been fully explained. As a result, the aim of this study was to examine DHA's anti-inflammatory and antioxidative actions in a mouse model of indomethacin-induced gastric injury. Oral administration of DHA (3, 10, 30, and 100 mg/kg) caused a reduction in indomethacin-induced gastric hemorrhagic lesions. We found that the gastroprotective effects of DHA treatment (100 mg/kg) were accompanied by decreases in several parameters: in leukocyte recruitment; gastric levels of myeloperoxidase; leukotriene B_4; intercellular adhesion molecule-1; tumor necrosis factor alpha; and nuclear translocation of nuclear factor-κB. Concurrently, we observed an improvement in antioxidant defenses produced by the increase in superoxide dismutase and glutathione activities but not catalase; in addition, a decrease in some oxidative damage markers such as malondialdehyde and carbonyl proteins in lipids and proteins was observed. Furthermore, resolvin D1 production and expression of free fatty acid receptor 4 were stimulated by DHA. Therefore, this study identified the antioxidant and anti-inflammatory actions of DHA as the main mechanisms involved in DHA's gastroprotective effects against indomethacin-induced gastric damage.

1. Introduction

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is limited by the extensive damage induced in the gastrointestinal (GI) tract (Bindu et al., 2013; Whittle, 2003). Inhibition of cyclooxygenase (COX) enzymes and subsequent suppression of gastric prostaglandin (PG) production has been considered the major reason for NSAID-induced gastric pathogenesis (Sinha et al., 2015; Yadav et al., 2012). In addition, during the past few decades, several studies have demonstrated that NSAIDs (such as indomethacin) generate gastric injury due to the production of reactive oxygen species (Bastaki and Wallace, 1999; Bindu et al., 2013); reactive oxygen species are associated with inflammatory process activation in damaged gastrointestinal tissue (Suleyman et al., 2010; Wallace, 2008).

The current prevention strategies for NSAID-associated gastropathy have not been completely effective (Wallace, 2013), as was shown in the case of proton pump inhibitors (PPIs). Despite the capability of these inhibitors to reduce gastric acid secretion, they have been reported to induce adverse effects (such as dysbiosis) as a consequence of chronic, long-term use (Wallace et al., 2011a).

Docosahexaenoic acid (DHA, C22:6), an omega-3 polyunsaturated fatty acid, which is abundant in fish oil (Holub, 2002), has shown

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neuroprotective, antinociceptive, antioxidative, and anti-inflammatory effects in experimental murine models such as those for hypoxia-ischemia brain and liver injury, formalin testing, and experimental colitis (Bento et al., 2011; Mayurasakorn et al., 2011; Nakamoto et al., 2010; Türkez et al., 2012). Recently, we reported that DHA, as a pure compound, has gastroprotective effect in the indomethacin-induced gastric injury model (Pineda-Peña et al., 2012). In this study, DHA did not reverse gastric prostaglandin E_2 (PGE_2) levels but partially prevented the increase in indomethacin-induced gastric leukotriene B_4 (LTB_4) levels (Pineda-Peña et al., 2012). Furthermore, DHA's protective effect has been associated with reduction of oxidative stress in brain (Hossain et al., 1999), liver (González-Périz et al., 2006), kidney (Ajami et al., 2013) and cardiac (Jahangiri et al., 2006) tissues by increasing the expression of superoxide dismutase (SOD) (Ajami et al., 2013), catalase (CAT) (Jahangiri et al., 2006), and glutathione levels (GSH) (Hossain et al., 1999). In addition, a decrease in malondialdehyde (MDA) was also observed (González-Périz et al., 2006). Several anti-inflammatory activities of DHA have been demonstrated: reduction of myeloperoxidase (MPO) in a murine ear inflammation model (Raederstorff et al., 1996); reduction of tumor necrosis factor alpha (TNF-α) in LPS- stimulated macrophages (Honda et al., 2015); reduction of intercellular adhesion molecule-1 (ICAM-1) expression in atherosclerosis (Huang et al., 2015); and prevention of nuclear factor-κB (NF-κB) activation in THP1-macrophages (Harvey et al., 2015; Yang et al., 2013). In addition, in a *fat*-1 transgenic mice model, eicosapentaenoic acid (EPA), another omega-3 polyunsaturated fatty acid, appeared to exert protective effects on the GI tract via activation of free fatty acid receptor 4 (FFA4 receptor) after NSAID-induced GI damage had occurred (Han et al., 2016).

However, the anti-inflammatory and antioxidative pathways have not been studied with respect to DHA's gastroprotective actions. Thus, the aim of this study was to evaluate the anti-inflammatory and antioxidative mechanisms of DHA in the indomethacin-induced gastric injury model.

2. Material and methods

2.1. Drugs and reagents

DHA (D2534), indomethacin (I7378), omeprazole (O104), and olive oil (1514), were purchased from Sigma Aldrich (Toluca, Mexico). Olive oil was utilized as the vehicle for DHA, indomethacin was dissolved in 5% $NaHCO_3$, and omeprazole was dissolved in 0.9% saline solution. All reagents were prepared prior to use.

2.2. Animals

Male Balb-c mice, weighing 20–25 g, were obtained from Centro de Investigación y de Estudios Avanzados (CINVESTAV) del Instituto Politécnico Nacional (Mexico City, Mexico) (Protocol number: 0184-03). All treatments for the animals, their care, and surgical procedures were performed in accordance with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) and the Bioethics Committee of ENMyH-IPN (registry number: ENMH-CB-139–2015) and were in compliance with international rules and standards on the care and use of laboratory animals. Sample size per group consisted of five to seven animals. Animals were fed with standard laboratory chow and tap water *ad libitum*. Mice were placed in cages with wire-net floors to minimize coprophagy and fasted 12 h prior to experimentation but were allowed free access to tap water while fasting.

2.3. Induction of gastric ulceration and assessment of gastric mucosal lesions

Mice were randomly divided into equal groups and treated via oral gavage as follows: 1.) Group 1, Control (olive oil); 2.) Groups 2, 3, 4, and 5, received a single administration of DHA (at doses of 3, 10, 30, and 100 mg/kg, respectively); 3.) Group 6, vehicle for DHA (olive oil) that was administered 2 h prior to indomethacin; 4.) Group 7, received omeprazole (30 mg/kg); and 5.) Group 8, vehicle for omeprazole (0.9% saline solution) 30 min prior to administration of the ulcerogenic agent. Five h after oral administration by gavage of the ulcerogenic agent (indomethacin 30 mg/kg) or the same volume of vehicle (5% $NaHCO_3$ for control), mice were anesthetized with ketamine (100 mg/kg) and xylazine (7.5 mg/kg) and killed. Stomachs were removed, opened along the greater curvature, and thoroughly rinsed with saline solution. The extent of the gastric-damaged area was scored blindly. For this, a picture of the fully extended stomach was taken; the length and width of each lesion was measured using ImageJ software (Version 1.45), and the total lesion area of the stomach (mm^2) was obtained for each mouse (Navarrete et al., 2005; Pineda-Peña et al., 2012; Wallace et al., 2011b, 2000). Based on the dose-response curve performed, we selected 100 mg/kg, p.o. of DHA for further analysis.

2.4. Histological study

For histological assessment, gastric tissue was excised and fixed with 10% formaldehyde in phosphate buffered saline (PBS) for 24 h. These tissues were then washed with tap water, dehydrated in alcohol, and embedded in paraffin. Sections of 4–5 μm were mounted on glass slides covered with silane. Hematoxylin and eosin staining was performed on each slide (Reyes-Gordillo et al., 2007), and slides were then examined under an optical microscope (Nikon Eclipse Slog) equipped with a high-resolution digital camera (Nikon Digital Sight DS-2mv).

2.5. Measurement of gastric MPO levels

Myeloperoxidase (MPO) tissue concentrations were determined employing a modified version of previously described methods (Jung et al., 2012; Seo et al., 2012; Yan et al., 2011). The MPO value was calculated by measuring the absorbance of samples at 620 nm and comparing them to a MPO standard (Yan et al., 2011).

2.6. Determination of gastric mucosal LTB_4, TNF-α, ICAM-1 and RvD1 levels

A sample of the corpus region of the stomach was excised, weighed, and added to a tube containing 1 ml of PBS (10 mmol/l; pH 7.4). The tissue sample was minced with scissors for 30 s and then placed in a shaking water bath (37 °C) for 20 min. The samples were centrifuged (9000*g*) for 1 min, and the supernatant was snap-frozen and then stored at −70 °C (Díaz-Triste et al., 2014; Wallace et al., 2000). The supernatant was used for determination of LTB_4, TNF-α, ICAM-1, and RvD1 levels by enzyme-linked immunosorbent assay (ELISA) using commercially available ELISA kits from Cayman Chemical Co. (Ann Arbor, MI, USA) and Thermo Fisher Scientific, Inc. (Waltham, MA, USA), according to the manufacturer's instructions. Values obtained were expressed per mg of tissue (Wallace et al., 2000).

2.7. Nuclear protein isolation and assay for NF-κB nuclear translocation

To obtain nuclear extracts from the corpus region of the gastric tissue to assay for nuclear translocation of NF-κB, samples from control and experimental sets of mice were utilized as previously described (Dimauro et al., 2012). Protein contents of the cytosolic and nuclear extracts were determined using the bicinchoninic acid protein assay, and the purity of the nuclear extract was determined by Western Blot for lamin-β1. The nuclear translocation of NF-κB was estimated using a commercial NF-κB (p65) Transcription Factor Assay Kit, which combines the principles of the electrophoretic mobility shift assay and ELISA as indicated by the manufacturer's instructions (Cayman Chemical Co., Ann Arbor, MI, USA).