European Journal of Pharmacology 697 (2012) 139–143

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

Docosahexaenoic acid, an omega-3 polyunsaturated acid protects against indomethacin-induced gastric injury

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ARTICLE INFO

Article history:
Received 14 July 2012
Received in revised form
20 September 2012
Accepted 21 September 2012
Available online 11 October 2012

Keywords:
Docosahexaenoic acid
Gastric injury
Indomethacin

ABSTRACT

Previous studies have shown gastroprotective effect of fish oil in several experimental models. However, the mechanisms and active compounds underlying this effect are not fully understood. Fish oil has several components; among them, one of the most studied is docosahexaenoic acid (DHA), which is an omega-3 long-chain polyunsaturated fatty acid. The aim of this study was to examine the gastroprotective effect of DHA as a pure compound in a rat model of indomethacin-induced gastric injury as well as elucidate some of the mechanism(s) behind DHA's gastroprotective effect. Indomethacin was orally administered to induce an acute gastric injury (3, 10 and 30 mg/kg). Omeprazol (a proton pump inhibitor, 30 mg/kg, p.o.) and DHA (3, 10, 30 mg/kg, p.o.) were gavaged 30 and 120 min, respectively, before indomethacin insult (30 mg/kg p.o.). Three hours after indomethacin administration, rats were sacrificed, gastric injury was evaluated by determining the total damaged area. A sample of gastric tissue was harvested and processed to quantify prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) levels by enzyme-linked immunosorbent assay. Indomethacin produced gastric injury in dose-dependent manner. DHA protected against indomethacin-induced gastric damage, and this effect was comparable with omeprazol's gastroprotective effect. DHA did not reverse the indomethacin-induced reduction of PGE_2 gastric levels. In contrast, DHA partially prevented the indomethacin-induced increase in LTB_4 gastric levels. This is the first report demonstrating DHA's gastroprotective effect as a pure compound. Furthermore, the results reveal that the gastroprotective effect is mediated by a decrease in gastric LTB_4 levels in indomethacin-induced gastric damage.

1. Introduction

Traditional non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin and indomethacin are widely used for the relief of pain and inflammation. However, their use is limited by their gastric and renal toxicity. NSAIDs-induced gastric damage is due in large part to cyclo-oxygenases (COXs) inhibition (Lanza, 1989). While COX-1 is involved in the biosynthesis of prostaglandins that regulate mucosal blood flow and epithelial mucus and bicarbonate secretion, prostaglandins derived from COX-2 are involved in the decrement of leukocyte adherence and re-epithelization of gastric cells (Wallace, 1997). Furthermore, COX-2 plays a very important role in ulcer healing (Wallace and Devchand, 2005). In fact, even with the introduction of selective COX-2 inhibitors (coxibs) gastric injury has not been abolished (Wallace and Vong, 2008). In light of these findings, there is a clinical need for therapies that prevent the gastric toxicity of NSAIDs.

Gastroprotective effects of fish oil have been reported in gastric ulcers induced by ethanol (Faust et al., 1989; Leung (1994)), aspirin (Faust et al., 1990; Bhattacharya et al., 2006), indomethacin (Güzel et al., 1995), dexamethasone (Manjari and Das, 2000), cold-restrain stress (Ulak et al., 1995) and pyloric ligation (Bhattacharya et al., 2006). Several mechanisms have been suggested to be involved in the gastroprotective effects of fish oil including a decrease in gastric acid secretion and lipid peroxidation as well as an increase in antioxidant enzymes during pylorus ligation and cold-restrain stress in rats (Bhattacharya et al., 2006).

Although several studies have shown fish oil's gastroprotective effect and have reported some potential mechanisms behind this effect, it is less understood which pure compounds of fish oil are responsible for this effect. Docosahexaenoic acid (DHA) is an omega-3 long-chain polyunsaturated fatty acid present in fish oil. DHA possesses several actions such as anti-inflammatory, neuroprotectitve and cardioprotective effects (Mayurasakorn et al., 2011; Saravanan et al., 2010). DHA also inhibits dextran sulfate

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http://dx.doi.org/10.1016/j.ejphar.2012.09.049

sodium-induced colitis in mice. Furthermore, DHA down regulates tumor necrosis factor alpha and interleukin 1-beta increased by colitis (Cho et al., 2011). Together, these findings suggest that DHA may be a pure compound of fish oil responsible for the gastroprotective actions.

Many substances have been proposed to be involved in the gastric damage induced by NSAIDs such as PGE_2 and leukotriene B_4. It has been demonstrated that PGE_2 increases mucus, bicarbonate and gastric blood flow and decreases acid secretion. Thus, an inhibition in the synthesis of prostaglandins, including PGE_2, by NSAIDs would block the gastro-protective actions of PGE_2. Furthermore, LTB_4 has been implicated in signal NSAID-induced leukocyte adhesion (Asako et al., 1992) and inhibition of synthesis of LTB_4 resulted in a significant reduction of NSAID-induced gastric injury (Santucci et al., 1994).

In this study, the aim was to examine the gastroprotective effect of DHA as a pure compound in an acute indomethacin-induced gastric injury model in the rat and to determine whether DHA modifies the gastric levels of PGE_2 and LTB_4 in rats with indomethacin-induced gastric damage.

2. Material and methods

2.1. Drugs

Docosahexaenoic acid (D2534), omeprazol (O104) and indomethacin (I7378) were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Animals

All the experiments were performed with female Wistar rats (180–220 g) obtained from Centro de Investigación y de Estudios Avanzados del IPN (Mexico, City). Procedures involving rats and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999) and in compliance with the international rules on care and use of laboratory animals. The sample size per group was five to seven animals. Rats were fed with standard laboratory chow and tap water *ad libitum*. The rats were placed in cages with wire-net floors and fasted 18 h before experimentation, but allowed free access to tap water while fasting.

2.3. Acute gastric injury induced by indomethacin

Groups of at least five rats received oral administration of indomethacin (3, 10, 30 mg/kg). The control group received the same volume of vehicle (sodium bicarbonate 0.5%). Three h later, rats were euthanized in a CO_2 chamber. The stomach was removed, and opened along the greater curvature. An observer, unaware of the treatment that the rats had received, measured the extent of area damage (mm^2) of each lesion. The damaged area was determined by measuring the width and the length of each lesion. The area of all lesions in the corpus of the stomach for each animal was calculated by summing the values and reported as gastric lesions (mm^2) (Wallace et al., 2000).

2.4. Gastroprotective effect of DHA on indomethacin-induced gastric injury

Rats received oral administration of olive oil (as vehicle of DHA, 0.1 ml/100 g) or three doses of DHA (3, 10 and 30 mg/kg, 0.1 ml/100 g). After 2 h, all rats were administered indomethacin to produce gastric mucosal lesions. Finally, gastric damage was measured as described above.

2.5. Gastroprotective effect of omeprazol on indomethacin-induced gastric injury

Rats received oral administration of saline solution (as vehicle of omeprazol, 0.1 ml/100 g) or omeprazol (30 mg/kg, 0.1 ml/100 g (Navarrete et al., 2005). Thirty minutes later, all rats received oral administration of indomethacin to induce gastric mucosal lesions. Finally, gastric damage was determined 3 h later as described above.

2.6. Measurement of endogenous levels of PGE_2 and LTB_4

Groups of at least five rats received olive oil or DHA (3, 10 and 30 mg/kg p.o.), and 2 h later groups were gavaged with indomethacin (30 mg/kg p.o.). Then, 3 h later, rats were sacrificed in a CO_2 chamber. The stomach was removed, a sample of the corpus region of the stomach was then excised, weighed, and added to a tube containing 1 ml of sodium phosphate buffer (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, the supernatant was snap-frozen, and then stored at −70 °C. The concentration of PGE_2 and LTB_4 in the supernatant was determined by enzyme-linked immunosorbent assay (PGE_2 and LTB_4 EIA kit Cayman Chemical Co.) (Wallace et al., 2000).

2.7. Statistical analysis

All data are expressed as mean ± S.E.M. Comparisons among control were performed using a one-way analysis of variance followed by Neuman–Keuls's test. $P \leq 0.05$ was considered as a statistically significant difference between means.

3. Results

Oral administration of indomethacin resulted in a dose-dependent gastric injury, where gastric damage was more evident at a dose of 30 mg/kg, (Fig. 1). Therefore, this dose of indomethacin was used to evaluate the gastroprotective effect of DHA.

Pre-administration of DHA decreased gastric area damage induced by indomethacin in a dose-dependent fashion (3, 10 and 30 mg/kg, p.o.) (Figs. 2, 3). This gastroprotective effect was statistically significant at all doses tested as compared to the effect produced by the vehicle administration (olive oil, Fig. 2). Furthermore, the gastroprotective effect of DHA at 30 mg/kg was not significantly different from that induced by the proton pump inhibitor, omeprazol (Fig. 4).

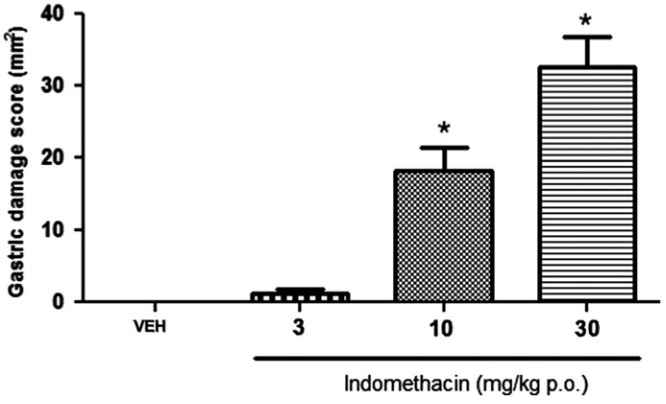

Fig. 1. Gastric injury induced by acute oral administration of indomethacin in the rat. Data are presented as mean ± S.E.M. (n=5–7) *$P \leq 0.05$ vs. vehicle (VEH).

In order to gain insight into the mechanisms behind DHA's gastroprotective effect, gastric levels of PGE_2 and LTB_4, substances previously reported to be involved in gastric injury were evaluated. Indomethacin decreased levels of PGE_2 in gastric tissue as compared with basal levels. Pre-treatment of DHA did not modify gastric PGE_2 levels in rats with indomethacin-induced gastric injury model (Fig. 5). In contrast, oral administration of indomethacin increased LTB_4 levels in gastric tissue as compared to the control group. Pre-treatment with DHA partially prevented indomethacin-induced increase of gastric LTB_4 levels (Fig. 6).

4. Discussion

The limited gastric safety offered by traditional NSAIDs has encouraged seeking new compounds to decrease their toxicity. The present study shows for the first time that DHA resulted in

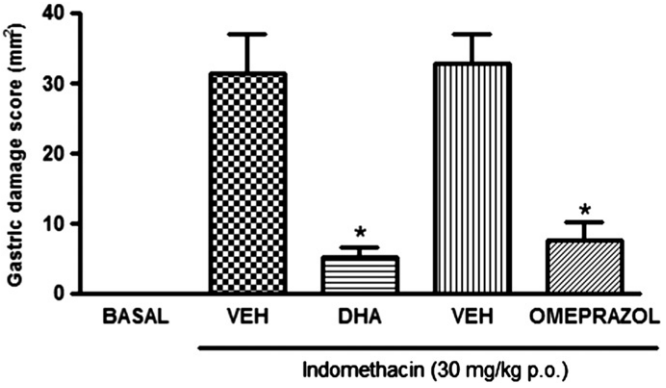

Fig. 4. Effect of DHA (docosahexaenoic acid, 30 mg/kg p.o.) and omeprazol (30 mg/kg p.o.) in the indomethacin-induced gastric injury model in rats. Data are presented as mean ± S.E.M. (n=5–7) $^*P \leq 0.05$ vs. respective vehicle (VEH).

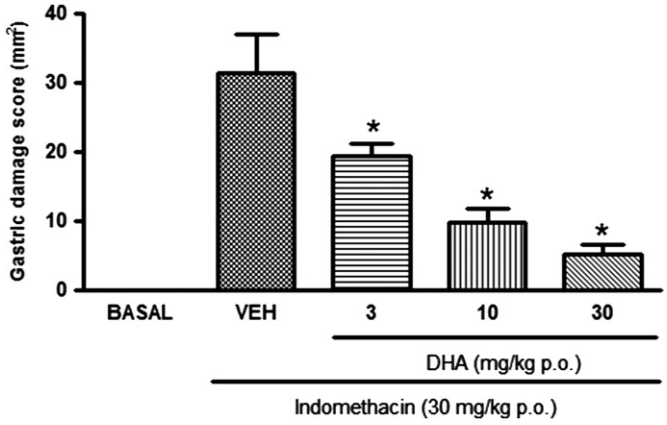

Fig. 2. Gastroprotective effect of DHA (docosahexaenoic acid) in the indomethacin-induced gastric injury model in rats. DHA was gavaged 120 min before indomethacin administration. Data are presented as mean ± S.E.M. (n=5–7) $^*P \leq$ 0.05 vs. vehicle (VEH).

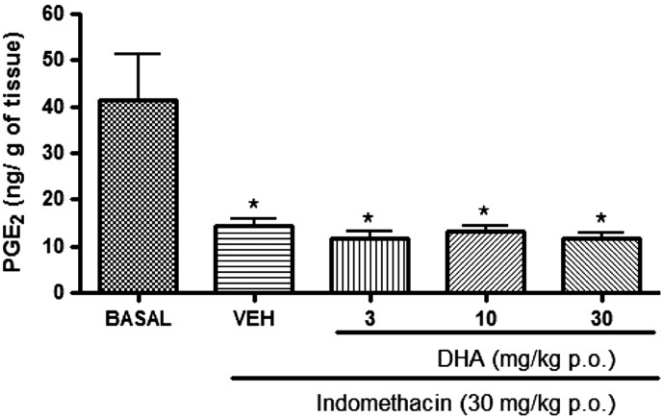

Fig. 5. Gastric prostaglandin E_2 (PGE_2) levels after DHA treatment in the indomethacin-induced gastric injury model in rats. Data are presented as mean ± S.E.M. (n=5–7) $^*P \leq 0.05$ vs. basal group.

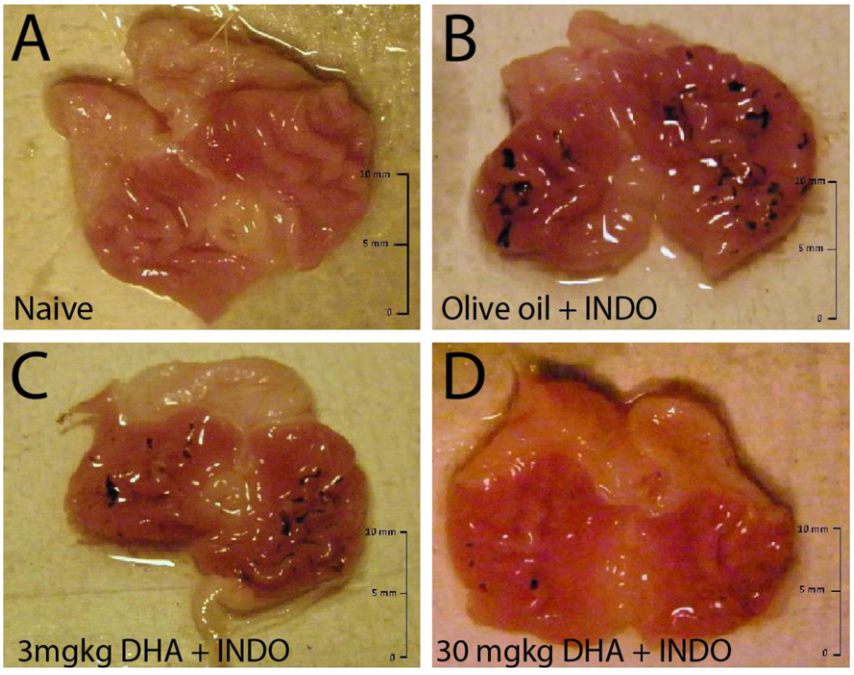

Fig. 3. Representative images of gastric lesions in the corpus of the stomach following different treatments. Naïve (panel A), olive oil + indomethacin (INDO) (panel B), DHA (3 mg/kg) + indomethacin (panel C), and DHA (30 mg/kg) + indomethacin (panel D).

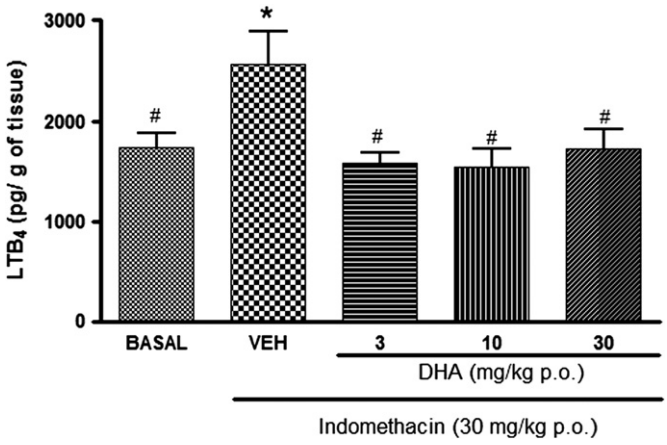

Fig. 6. Gastric leukotriene B_4 (LTB_4) after DHA treatment in the indomethacin-induced gastric injury model in rats. Data are presented as mean ± S.E.M. ($n=5–7$) $^{*}P \leq 0.05$ vs. basal group, $^{\#}P \leq 0.05$ vs. vehicle (VEH).

gastric mucosa protection during indomethacin-induced gastric injury. Furthermore, this study reveals that DHA's gastroprotective effect is mediated by a partial blockade of the indomethacin-induced increase of LTB_4.

Fish oil's gastroprotective effect has been reported after several insults to the gastric mucosa (Bhattacharya et al., 2006; Manjari and Das, 2000). Fish oil is rich in polyunsaturated fatty acids like eicosapentaenoic acid and docosahexaenoic acid. Composition of polyunsaturated fatty acids in fish oil depends on the source and the alimentation that the fish have received. The proportion of polyunsaturated fatty acids found in rat plasma after a diet rich in fish oil was 9.57% for DHA and 18.78% for EPA (Manjari and Das, 2000).

While several studies (Faust et al., 1990; Bhattacharya et al., 2006) have reported gastroprotective effect after chronic treatment of fish oil, the present results demonstrate that acute DHA administration induced gastroprotection in the indomethacin-induced gastric injury model in the rat. Moreover, the effect produced by the highest dose of DHA was not significantly different from the gastroprotective effect exhibited by the proton pump inhibitor, omeprazol. Recently, it has been reported that DHA is a safe compound (Fedorova-Dahms et al., 2011), in contrast to proton pump inhibitors that may exacerbate NSAID-induced gastrointestinal damage (Wallace et al., 2011). These results together suggest that DHA may be a potential alternative for prevention of the gastric injury induced by NSAIDs.

NSAIDs-induced gastric damage is due in large part to (COXs) inhibition, which results in inhibition of synthesis of prostaglandins. Prostaglandins participate in the signal of gastric protective factors; PGE_2 is involved in the increase of mucus and gastric blood flow secretion. NSAIDs-induced gastric mucosal injury includes inhibition of mucosal prostaglandin synthesis (Wallace and Devchand, 2005).

LTB_4 induces inflammation, leukocyte chemotaxis and adherence (Wallace, 1997). It has been shown that NSAIDs-induced gastric injury, such as from treatment with indomethacin, results in an increase in LTB_4-dependent leukocyte adherence (Asako et al., 1992). LTB_4 effects on gastric and mesenteric microcirculation lead to an increase in leukocyte adherence, and at the same time, allow the liberation of oxygen-derived free radicals and proteases (Wallace, 1997). Furthermore, an increase in the LTB_4-dependent neutrophil adherence to the vascular endothelium could obstruct capillaries, resulting in a reduction in gastric mucosal blood flow and thereby predisposing the mucosa to gastric injury (Wallace, 1997). Our results reveal that the gastroprotective effect of DHA was associated with a significant reduction of gastric LTB_4. However, LTB_4 reduction was not dose-dependent, while DHA-induced gastroprotective effect was dose-dependent. Whether DHA is modifying the leukocyte adherence and/or gastric mucosal blood flow is unclear but is the subject of future investigations.

DHA *in vitro* generates a new family of pro-resolution mediators named resolvins. DHA is enzymatically converted to resolvin D1 (RvD1) in a multistep reaction that proceeds through a transcellular biosynthetic route during endothelial cell–neutrophil interactions (Serhan et al., 2000). RvD1 produces anti-inflammatory and antinociceptive effects in several animal models (Ji et al., 2011). RvD1 stops neutrophil recruitment, regulates cytokine and chemokine expression and decreases LTB_4 functions (Norling et al., 2012). RvD1's effect has been explored in dextran sulfate sodium-induced colitis where RvD1 reduced colonic cytokine levels (Bento et al., 2011). RvD1 may also contribute to DHA gastroprotective effects. Further studies need to be performed to determine whether the gastroprotective effect of acute DHA is associated with changes in the expression of RvD1.

The present results show that acute DHA administration blocks the indomethacin-induced increase of LTB_4 levels. A key question is how DHA exerts this effect? Omega-3 polyunsaturated fatty acids, such as DHA may exert beneficial effects by competing with omega-6 polyunsaturated fatty acids such as arachidonic acid for the production of lipid inflammatory mediators like leukotriene (Gil, 2002). Furthermore, it has been shown that human ingestion of fish oil leads to a decrease in LTB_4 and an increase in LTB_5, a weak inducer of inflammation and a weak chemotactic agent (Simopoulos, 2002).

The present study has some potential limitations. While the present results show that acute DHA prevents the gastric toxicity of indomethacin, it is unclear whether chronic DHA will also block the gastric side effects of indomethacin and other NSAIDs. Furthermore, future studies are needed to determine whether the co-administration of DHA with NSAIDs modifies the anti-inflammatory and analgesic effects of NSAIDs.

In conclusion, this is the first report that demonstrates the gastroprotective effect that DHA exhibits as a pure compound in indomethacin-induced gastric injury. The role of LTB_4 is important in the gastroprotective effect of DHA; however, other mediators may be involved in the DHA gastroprotective effect. Understanding the mechanisms behind DHA's gastroprotective effect may allow the development of therapies that may be co-administered with NSAIDs to prevent their gastric toxicity without modifying their analgesic and anti-inflammatory effects.

Acknowledgment

Authors acknowledge the support of Instituto Politécnico Nacional with the project SIP-20121164. Elizabeth Arlen Pineda-Peña is a CONACyT fellowship.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ejphar.2012.09.049.

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