Leukotriene B_4 limits the effectiveness of fish oil in an animal model of asthma

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Highlights

- Unraveling the action of fish oil in the lungs of asthmatic rats.
- Fish oil modifies inflammatory pathways with enzymatic specificity.
- Leukotriene B_4 remains unchanged with fish oil supplementation in asthmatic rats.

ABSTRACT

This study aimed to evaluate the levels of eicosanoids derived from arachidonic acid (ARA) in the lungs of asthmatic rats supplemented with fish oil. The present data gives insight into the action of fish oil in asthma, related to its inability to modify the contractile capacity of tracheal smooth muscle reported previously in a model of asthma in rats. Male Wistar rats were supplemented daily with 1 g of fish oil/kg of body weight for 21 days. They were exposed to ovalbumin (OVA) after previous sensitization with OVA to induce asthma. Pulmonary levels of five eicosanoids were measured using immunoassay kits: PGE_2, TXB_2, LTB_4, LXA_4, and 8-iso $PGF_{2\alpha}$. In asthmatic rats, supplementation with fish oil resulted in lower concentrations of lung eicosanoids produced by cyclooxygenase-2 and 15-lipoxygenase: PGE_2, TXB_2, and LXA_4, respectively. Fish oil supplementation also decreased the non-enzymatically produced eicosanoid 8-iso $PGF_{2\alpha}$. Fish oil supplementation did not affect LTB_4, a metabolite of 5-lipoxygenase. The limited efficacy of fish oil supplementation in asthmatic rats is associated with a lack of action in reducing the levels of LTB_4 in the lungs. Thus, fish oil differentially modulates the concentrations of eicosanoids derived from ARA via specific pathways in an animal model of asthma.

Keywords: fish oil, asthma, eicosanoid, leukotriene

Introduction

In allergic asthma, exposure to an allergen triggers immediate acute airway inflammation and bronchoconstriction. This condition involves the release of eicosanoids derived from the n-6 fatty acid arachidonic acid (ARA), and their actions affect the functional response of bronchial smooth muscles [1,2]. In the pulmonary microenvironment, these bioactive molecules lead to the main feature of asthma: bronchial hyper-reactivity [3,4,5,6]. Among the various eicosanoids, the presence of 4-series leukotrienes (LTs) has a remarkable effect: they are considered the central mediators of airway hyper-responsiveness and asthmatic bronchoconstriction [7,8,9,10]. The formation of LTB_4 from ARA is via the 5-lipoxygenase (5-LOX) pathway in the lungs [5,7,11]. There is significant literature on the role of LTB_4 in triggering contractile processes in the smooth muscles of the airways [7,12,13,14,15]. LTB_4 activates specific receptors of the airway smooth muscle [16], and it is likely related to airway hyper-responsiveness in animal models of asthma [11]. Increased levels of LTB4 promote asthma exacerbation, edema, and mucus secretion, which also contribute to airway obstruction [11,12,13].

Other eicosanoids produced from ARA by different pathways also initiate the process of contraction of airway smooth muscle [1,17,18,19]. For example, the cyclooxygenase (COX)-derived eicosanoids thromboxane A2 (TXA2), prostaglandin (PG) D2 (PGD2) and PGF2 [19], and F2 isoprostanes derived by non-enzymatic oxidation of ARA (e.g., 8-isoPGF2α) [20] are all involved. However, these molecules are less potent bronchoconstrictors than the LTs [15,17]. Another eicosanoid involved is lipoxin A4 (LXA4) [21], a product of the 15-LOX pathway. This molecule acts to suppress inflammation and activate resolution and recovery processes [22,23]. LXA_4 works

by regulating inflammatory responses in association with resolvins and maresins produced by n-3 fatty acids [6].

The production of LTs is relatively resistant to corticosteroids [14,24], which are considered the first-line drugs of asthma treatment [25,26,27,28]. Thus, the study of treatments with a potential effect on the production of the different eicosanoids could provide an alternative for asthma control [6]. Fish oil is a source of n-3 fatty acids, and when used as a supplement it can reduce the production of some ARA-derived eicosanoids and decrease leukocyte chemotaxis in asthmatics [29,30,31,32,33,34]. Still, the efficacy of fish oil supplementation in asthma remains inconsistent [35,36], and clinical data on the effect of fish oil supplements in asthma have been equivocal [37, 38, 39, 40, 41, 42, 43]. Fish oil has a limited impact on airway smooth muscle function in asthmatic rats [43]. This lack of fish oil efficacy may be related to a limited effect on the lung production of inflammatory mediators that actively participate in the smooth muscle hyper-responsiveness of the airways. Thus, this study aimed to evaluate the effects of fish oil supplementation on eicosanoids derived from ARA in the lungs of asthmatic rats to elucidate a possible mechanism for the limited impact of fish oil in airway hyper-responsiveness in these animals. The study hypothesizes that the inconsistent efficacy of fish oil is related to LT levels in asthmatic rat lungs.

Materials and Methods

Animals

Male Wistar rats aged 8 wk and weighing 220±30 g were purchased from the animal house of Universidade Federal do Paraná, Curitiba, Brazil. Rats were randomly divided into four experimental groups: non-asthmatic control (C, n=16), non-asthmatic supplemented with fish oil (FO, n=16), asthmatic (A, n=16), and asthmatic supplemented with fish oil (AFO, n=16). Rats were allowed free access to water and food (52% by weight of carbohydrates, 21% by weight of proteins, and 4% by weight of total lipids; Nuvilab CR1, Nuvital Nutrientes Ltda, Curitiba, Paraná, Brazil). Rats were maintained at a room temperature of approximately 23°C with 55% relative humidity and a 12 h inverted light/dark cycle. The Institutional Animal Ethics Committee, which acts according to the Brazilian College on Animal Experimentation (COBEA) guidelines, approved all procedures (protocol number 468).

Supplementation and asthma induction

Fish oil (Herbarium®, Colombo, Paraná, Brazil) was administered daily into the mouth of rats in the FO and AFO groups using a micropipette at a dose of 1 g/kg of body weight for 21 days. For the allergic sensitization, rats from groups A and AFO received a subcutaneous injection of ovalbumin (OVA): the solution contained 1 mg/mL OVA (Grade II, Sigma, St. Louis, Missouri, USA) complexed with 20 mg of the adjuvant $Al(OH)_3$ (Sigma, St. Louis, Missouri, USA) in phosphate-buffered saline (PBS). The solution was administered five days after starting fish oil supplementation and was repeated seven days later. C and FO rats received only the adjuvant in PBS. Rats were exposed to an aerosol of 5% OVA diluted in sterile PBS for 30 min on three consecutive days before the end of supplementation, using an ultrasonic nebulizer (Ultraneb) as a pulmonary challenge (43).

Lung collection

Twenty-four hours after the final OVA challenge, rats received an intraperitoneal injection of thiopental (20 mg/kg) (Sigma, St. Louis, Missouri, USA) and were euthanized by an injection of potassium chloride (2 mmol/kg) (Sigma, St. Louis, Missouri, USA). After thoracotomy, the lower left lobe of the lungs was removed, washed in PBS and frozen in liquid nitrogen before storage at -80°C for later determination of eicosanoid concentrations.

Determination of eicosanoid concentrations

PGE_2, TXB_2, LTB_4, and 8-iso $PGF_{2\alpha}$ concentrations were determined using enzyme immunoassay kits from Cayman (Cayman Chemical Co., MI, USA). LXA_4 concentration was determined using an EIA kit from Oxford Biomedical Research (Oxford, UK). Lung tissue was homogenized (micro-homogenizer IKA T10 basic, Ultra Turrax) at a concentration of 25 mg/mL in homogenization buffer (0.1 M phosphate, pH 7.4, containing 1 mM EDTA and 10 μM indomethacin for PGE_2, TXB_2, LTB_4 and LXA_4 assays; 0.1 M phosphate, pH 7.4, containing 1 mM EDTA and 0.005% butylated hydroxytoluene (BHT) for 8-iso $PGF_{2\alpha}$ assay), and centrifuged for 10 min at 9,500 r.p.m. The dilutions of the supernatants were 1:50 for PGE_2, LTB_4, and 8-iso $PGF_{2\alpha}$, 1:500 for TXB_2, and 1:5 for LXA_4. In the next step, the supernatant, the conjugated eicosanoid-acetylcholinesterase complex, and the specific antiserum were mixed for incubation. The 96-well plates were pre-coated with mouse anti-rabbit immunoglobulin G antibodies. After the incubation period (60 min or 18 h or overnight) at the temperature (room temperature or 4ºC) recommended for each kit, the enzyme-substrate was added and incubated for 30 to 120 min in the dark at room temperature. Plates were read on a microplate reader (Multiskan EX, Thermo Labsystems) at 650 nm for PGE_2, TXB_2, LTB_4, and 8-iso $PGF_{2\alpha}$ or 405 nm for LXA_4. Eicosanoid concentrations were calculated from a standard curve, according to the kit protocol, and corrected for mg of lung tissue.

Statistical analysis

Data are expressed as mean ± SEM. One-way ANOVA was used to analyse the data with post hoc comparisons between groups using Tukey's test. Analyses were conducted using Prism 8.4.2 software (GraphPad, San Diego, CA, USA). In all cases, differences were considered significant for $p<0.05$.

RESULTS

Lung eicosanoid concentrations are shown in Table 1. Asthma significantly increased the lung concentration of all five ARA-derived eicosanoids measured (A versus C; $p < 0.05$). Fish oil supplementation significantly decreased lung concentrations of TXB_2 and 8-iso $PGF_{2\alpha}$ in non-asthmatic rats (FO versus C; $p < 0.05$). Fish oil supplementation resulted in significantly lower lung concentrations of PGE_2, TXB_2, 8-iso $PGF_{2\alpha}$, and LXA_4 in asthmatic rats (AFO versus A; $p < 0.05$). However, fish oil did not significantly alter lung LTB_4 concentration in asthmatic (AFO versus A) or non-asthmatic (FO versus C) rats.

TABLE

Table 1. Lung eicosanoid concentrations (pg/mg) from asthmatic and non-asthmatic rats supplemented or not with fish oil.

	C	FO	A	AFO
Eicosanoid				
PGE_2	189.9±23.8 (n=11)	155.7±11.8 [b] (n=13)	265.8±13.6 [a c] (n=12)	182.0±7.1 [b] (n=15)
TXB_2	1207.0±89.0 (n=15)	642.7±85.9 [a b] (n=15)	1619.0±114.1 [a c] (n=15)	1031.0±81.2 [b c] (n=15)
LTB_4	39.8±5.4 (n=15)	47.5±5.9 (n=13)	73.6±7.5 [a] (n=15)	62.6±5.3 (n=15)
8-iso $PGF_{2\alpha}$	45.3±3.8 (n=12)	31.4±2.6 [a b] (n=13)	56.7±2.1 [a] (n=12)	33.9±1.3 [a b] (n=14)
LXA_4	0.032±0.001 (n=12)	0.026±0.003 (n=13)	0.122±0.015 [a] (n=10)	0.031±0.002 [b] (n=15)

[a] $p<0.05$ vs. C; [b] $p<0.05$ vs. A; [c] $p<0.05$ vs. FO.

DISCUSSION AND CONCLUSIONS

This study was designed to elucidate the effect of fish oil on the production of ARA-derived eicosanoids in the lungs of OVA-induced asthmatic rats in order to understand why fish oil did not modify the contractile capacity of tracheal smooth muscle in this model of asthma reported previously [43]. Fish oil is known to exert anti-inflammatory actions [44, 45, 46] and the assumed mechanism is the modification of the pattern of eicosanoids being produced from ARA, including LTB4 [46]. In the present work, fish oil supplementation decreased PGE2 and TXB2 in lungs obtained from asthmatic rats. These two mediators are derived from ARA via the COX pathway. Likewise, the fish oil group had decreased levels of 8-iso $PGF_{2\alpha}$ and LXA_4 in the lungs from asthmatic rats. These

metabolites are produced by non-enzymatic oxidation of ARA and via the 15-LOX pathway, respectively. The reduction in the levels of these four eicosanoids is most likely related to the incorporation of the biologically active n-3 fatty acids present in fish oil: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are incorporated into inflammatory cell membranes decreasing the relative content of ARA [47,48]. We previously reported that fish oil supplementation results in increased EPA and DHA and decreased ARA in lung tissue from these rats [49]. Thus, there is less substrate available (i.e. ARA) to produce the eicosanoids measured in the current study. Furthermore, the altered balance between the ARA and the n-3 fatty acids creates a competition for different enzymes involved in eicosanoid synthesis such as COX-2 [44,45,50,51,52]. These effects likely explain why PGE_2, TXB_2, LXA_4 and 8-iso $PGF_{2\alpha}$ concentrations are lower in the rats receiving fish oil.

Our findings demonstrate an increase in lung 8-iso $PGF_{2\alpha}$ in asthmatic animals not receiving fish oil. This bioactive molecule is closely related to oxidative stress observed in asthma [20,49]. With fish oil supplementation, 8-iso $PGF_{2\alpha}$ decreased in parallel with the before mentioned PGE_2, TXB_2, and LXA_4. The results are in accordance with the study of Yin et al. [16], which found lower levels of 8-iso $PGF_{2\alpha}$ in the lungs obtained from OVA-induced asthmatic animals with fish oil supplementation. The 2-series thromboxanes and isoprostanes have relevant roles in asthmatic inflammation [20] and they were significantly reduced in this study after fish oil supplementation. These mediators increase leukocyte chemotaxis, mucus secretion, and bronchial hyper-responsiveness to various stimuli and contribute to the obstruction of the airways seen in asthma [16,17,19,20]. However, the hyper-responsiveness of airway smooth muscle depends not only on these mediators but on the levels of other potent bronchoconstrictors particularly LTB_4.

PGE_2 induces the production of LXA_4 [1,6,19]. Thus, the observed decrease in the concentration of PGE_2 may also be relevant to the lower concentration of LXA_4 [53]. In other words, lower LXA_4 may not only relate to reduced ARA availability but to the lower PGE_2 production. The reduction in LXA_4 may be undesirable in the context of hyperinflammation, since it is anti-inflammatory and inflammation resolving. This points to a complex interplay between the eicosanoid mediators in determining the ultimate physiological and clinical outcome.

Increased synthesis of 4-series LTs is an essential factor in the acute inflammatory response observed in asthma. As already stated, LTs are the major eicosanoids participating in airway hyper-responsiveness and bronchoconstriction [9,10,11,12]. In the current study, fish oil did not reduce the concentration of LTB_4 in lungs of asthmatic rats. LTB_4 is the most potent eicosanoid involved in pulmonary smooth muscle contractile function. These observations suggest that the synthesis of LTs in asthma depends not only on the availability of the substrate ARA, which is diminished with fish oil administration, but also on other factors. For example, there may be other components of fish oil that affect COX and 15-LOX but not 5-LOX activity and/or expression.

In vitro and in vivo studies have shown that inhibition of the COX pathway could enhance the generation of LTs by increasing the substrate supply for 5-LOX [52,54] and can boost 5-LOX/COX ARA metabolite ratios [52]. In addition, PGE2 inhibits 5-LOX [55,56,57] and so, once again, the effect of fish oil on PGE_2 production may have permitted continued production of LTB_4, again highlighting the complex interplay between these metabolites. This finding is important because it may explain the lack of effectiveness of fish oil in this model previously demonstrated [43]. This effect could limit the clinical application of fish oil in people with asthma.

In conclusion, fish oil decreased the concentration of several inflammatory eicosanoids derived from ARA via COX and non-enzymatic pathways in the lung of asthmatic rats and reduced the concentration of the pro-resolving mediator LXA_4. However, the concentration of LTB_4, also derived from ARA by the 5-LOX pathway, was not changed. Thus, fish oil differentially modulates the concentrations of eicosanoids derived from ARA via specific pathways in an animal model of asthma.

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