Docosahexaenoic Acid and Other Dietary Polyunsaturated Fatty Acids Suppress Leukotriene Synthesis by Mouse Peritoneal Macrophages

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The efficacy of individual ω-3 polyunsaturated fatty acids (PUFA) in altering eicosanoid synthesis in peritoneal macrophages was studied by feeding mice for 10 days a diet containing 2 wt % fat, which included 0.5 wt % ethyl esters of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or linolenic acid (LNA). Upon stimulation with calcium ionophore A23187, macrophages from these animals produced significantly lower amounts of leukotriene C₄, leukotriene B₄, and 12-hydroxyeicosatetraenoic acid, prostaglandin E₂ and 6-keto prostaglandin F₁α, compared with those obtained from animals on the diets containing olive oil or safflower oil. The decrease in leukotriene synthesis was similar in the animals fed DHA, EPA or LNA diets. This depression of eicosanoids by DHA and EPA was associated with decreased levels of arachidonic acid (AA); however, LA that altered eicosanoids did not have the same effect on AA levels.

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The role of leukotrienes in inflammatory processes is well documented (1–3). Leukotriene B₄ (LTB₄) is a powerful chemotactic agent, but leukotriene C₄ (LTC₄) and other peptidoleukotrienes enhance vasopermeability and exudation of plasma cells (3–5). Excess or imbalanced production of these leukotrienes may exacerbate pathophysiological states like inflammation, asthma, arthritis and psoriasis (3,4,6,7). Therefore, the regulation of leukotriene production is vital in controlling inflammation and preventing pathophysiological states.

Leukotriene production can be inhibited by agents such as eicosatetraynoic acid, nortrihydroguaiaretic acid, BW755C, esculetin or gossypol (3,4,8,9). Certain dietary polyunsaturated fatty acids (PUFA), especially the ω-3 (n-3) PUFA series in fish oils, may modulate leukotriene synthesis and possibly ameliorate inflammatory conditions (10–13). Though fish oils contain both eicosapentaenoic acid (EPA), 20:5n3, and docosahexaenoic acid (DHA) 22:6n3 as the major n-3 PUFA components, the modulating effects of dietary fish oil on eicosanoid synthesis have been generally attributed to EPA, although the effect of DHA is equivocal (13–15). It has been reported that DHA may not affect leukotriene synthesis at the lipoxygenase level in human neutrophils (15) and RBL-1 cell homogenates (12). However, recently we demonstrated that mouse macrophages, enriched with DHA in vitro, produced significantly lower amounts of leukotrienes, compared with the control cells, indicating that DHA can significantly reduce leukotriene synthesis by altering the arachidonic acid (AA) substrate pools utilized for leukotriene synthesis (16).

In order to determine if similar effects can be induced in vivo, mice were fed a diet enriched with DHA. To compare the efficacy of DHA with other unsaturated fatty acids, groups of mice also were fed diets enriched with EPA (20:5n3), linolenic acid (LNA) (18:3n3), olive oil (18:2n6) or safflower oil (18:2n6).

MATERIALS AND METHODS

Materials. Calcium ionophore A23187 (lot 105f-4014), reduced glutathione and cysteine hydrochloride were purchased from Sigma Chemical Company (St. Louis, MO). Ethyl esters of linolenic acid (>99.5% pure) and docosahexaenoic acid (<99.5% pure) and a preparation of eicosapentaenoic acid ethyl esters (53% EPA, 7% docosapentaenoic acid, 13% DHA) were purchased from NuChek Prep (Elysis, MN). Commercial edible olive oil and safflower oil were obtained locally. Leukotriene standards were obtained from Merck Frosst (Montreal, Canada). Radioimmunoassay kits for quantification of prostaglandins (prostaglandin E₂ and 6-keto prostaglandin F₁α) were purchased from Seragen, Inc. (Boston, MA). Analytical grade solvents were used for the extraction of lipids and eicosanoids.

Animals. Male mice (CD-1 type) weighing 18–20 g were purchased from Charles River (Wilmington, MA) and housed five per cage. A 12 hr light-dark cycle and a temperature of 22°C was maintained in the room.

Diets. Fat-free diet was purchased from ICN Nutritional Biochemicals (Cleveland, OH). All diets were thoroughly mixed with 1.5 wt % olive oil and then supplemented with an additional 0.5 wt % olive oil (Diet A), 0.5 wt % safflower oil (Diet B), 0.5% wt % ethyl ester of LNA (Diet C), 0.5 wt % ethyl ester of EPA (Diet D) and 0.5 wt % DHA (Diet E). The diets were thoroughly mixed after the addition of oils, and small amounts (35 g) were transferred to Whirlpak plastic bags, flushed with nitrogen and stored at 4°C in the dark. The mice received fresh diet every day. There was no oxidation of dietary fats as measured by thiobarbituric acid reactive materials and also by the analysis of fatty acids (17). The fatty acid composition of the diets is shown in Table 1. Mice were fed a fat-free diet for one week to reduce endogenous ω-3 fatty acids and facilitate the rapid uptake of dietary fatty acid esters fed to the animals. The animals on a fat-free diet for one week did not show any symptoms of essential fatty acid deficiency and did not accumulate 20:3n-9 fatty acid. The net production of eicosanoids by macrophage from lab chow-fed animals were: Prostaglandin E₂ (PGE₂) 5.2 ± 0.96 ng/μg DNA and 6 keto Prostaglandin F₁α (6 keto PGF₁α) 32.3 ± 3.16 ng/μg DNA. The corresponding values for macrophages from fat-free-fed animals for one week were PGE₂ 5.45 ± 0.9 ng/μg DNA.
Fatty acid analyses. Because insufficient macrophage material was available for fatty acid analyses, spleen tissue which changes in a manner similar to macrophages in response to fish oil (26-28), were analyzed to monitor changes after feeding experimental diets.

Mice were killed by ether asphyxiation and the spleens were immediately removed and flushed with a buffer, 0.25 M sucrose containing 10 mM Tris and 1 mM EDTA buffer, pH 7.4. After removing the connective tissues, the spleens were weighed, minced using scissors and homogenized at 4°C in 5 vol of 0.05 M Tris HCl buffer, pH 7.4, in a polytron (Kinematics, Lucerne, Switzerland) for 60 sec in 2 × 30 sec bursts at setting 4, followed by a 20 sec burst with an Ultrasonics Model W-10 ultrasonic sonicator (Ultrasonics, Plainview, NY). The homogenate was collected after centrifuging at 600 g for 15 min in a Sorval RC5B to remove cell debris and unbroken cells. Lipids were extracted from spleen homogenates by the method of Bligh and Dyer (29). The phospholipids were separated from neutral lipids on HPTLC plates using chloroform/methanol (80:20, v/v). The lipids were saponified with 0.5 N KOH in methanol and the fatty acids were methylated with diazomethane. The fatty acid methyl esters were separated using a Hewlett-Packard 5880A gas chromatograph (Hewlett-Packard, Avondale, PA) with hydrogen as carrier gas. Fatty acids were identified by retention times compared with authentic standards, by their UV absorption spectra obtained by a diode array spectrophotometric detector and were quantified by their absorbances using extinction coefficients of authentic standards (16).

Prostaglandin analysis. Macrophages were stimulated with calcium ionophore A 23187 (0.5 μg/ml) for 2 hr at 37°C. Prostaglandins were extracted from the medium in three volumes of ethyl acetate and quantified by radioimmunoassay (26). The PGE₂ antiserum had a cross reactivity of less than 1% with TXB₂, 6-keto PGF₁α, HETE and AA. The 6-keto PGF₁α antiserum had a cross reactivity of 0.6% with PGE₂ and less than 0.1% with TXB₂, HETE and AA.

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Statistical methods. Results were statistically evaluated by Statistical Analysis System using the General Linear Models procedure and the least significant difference mean-separation determination (SAS Institute, Chapel Hill, NC) (31).

RESULTS AND DISCUSSION

The amounts of diet consumed (ca. 5 g/day/mouse) and the weight gains (6.1 ± 0.3 g/mouse in 10 days, mean ± SD, n = 10 per group) by the animals were similar in all dietary groups.